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(57) Abstract

This invention provides isolated nucleic acid molecules, proteins, monoclonal antibodies, pharmaceutical compositions, transgenic animals, methods of treatment, methods of screening, and methods of diagnosis for both the GABA transporter and taurine transporter.

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DNA ENCODING TAURINE AND GABA TRANSPORTERS AND USES THEREOF

This application is a continuation-in-part of U.S. Serial No. 847,742, filed March 4, 1992 the contents of all of which are hereby incorporated by reference into the subject application.

Background of the Invention

Throughout this application various publications are referred to by partial citations within parenthesis. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications, in their entireties, are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Chemical neurotransmission is a multi-step process which involves release of neurotransmitter from the presynaptic terminal, diffusion across the synaptic cleft, and binding to receptors resulting in an alteration in the electrical properties of the postsynaptic neuron. For most neurotransmitters, transmission is terminated by the rapid uptake of neurotransmitter via specific, high-affinity transporters located in the presynaptic terminal and/or surrounding glial cells (29). Since inhibition of uptake by pharmacologic agents increases the levels of neurotransmitter in the synapse, and thus enhances synaptic transmission, neurotransmitter transporters provide important targets for therapeutic intervention.

The amino acid GABA is the major inhibitory neurotransmitter in the vertebrate central nervous system and is thought to serve as the neurotransmitter at approximately 40% of the synapses in the mammalian brain (13,28). GABAergic transmission is mediated by two classes of GABA receptors. The more prevalent is termed $GABA_A$, which is a multi-subunit protein containing an intrinsic ligand-gated chloride channel in addition to binding sites for a variety of neuroactive drugs including benzodiazepines and barbiturates (35,73). In contrast, $GABA_B$ receptors couple to G-proteins and thereby activate potassium channels (2,35) and possible alter levels of the second messenger cyclic AMP (35). Positive modulation of $GABA_A$ receptors by diazepam and related benzodiazepines has proven extremely useful in the treatment of generalized anxiety (77) and in certain forms of epilepsy (57).

Inhibition of GABA uptake provides a novel therapeutic approach to enhance inhibitory GABAergic transmission in the central nervous system (36,62). Considerable evidence indicates that GABA can be taken up by both neurons and glial cells, and that the transporters on the two cell types are pharmacologically distinct (15,36,62). A GABA transporter with neuronal-type pharmacology designated GAT-1 has previously been purified and cloned (21), but the molecular properties of other GABA transporters including glial transporter(s) have not yet been elucidated. We now report the cloning of two additional GABA transporters (GAT-2 and GAT-3) with distinct pharmacology and localization, revealing previously unsuspected heterogeneity in GABA transporters.

Taurine (2-aminoethane sulfonic acid) is a sulfur-containing amino acid present in high concentrations in mammalian brain as well as various non-neural tissues. Many functions have been ascribed to taurine in both the nervous system and peripheral tissues. The best understood (and phylogenetically oldest) function of taurine is as an osmoregulator (26,75). Osmoregulation is essential to normal brain function and may also play a critical role in various pathophysiological states such as epilepsy, migraine, and ischemia. The primary mechanism by which neurons and glial cells regulate osmolarity is via the selective accumulation and release of taurine. Taurine influx is mediated via specific, high-affinity transporters which may contribute to efflux as well. Since taurine is slowly degraded, transport is an important means of regulating extracellular taurine levels.

Taurine is structurally related to the inhibitory amino acid γ-aminobutyric acid (GABA) and exerts inhibitory effects on the brain, suggesting a role as a neurotransmitter or neuromodulator. Taurine can be released from both neurons and glial cells by receptor-mediated mechanisms as well as in response to cell volume changes (64). Its effects in the CNS may be mediated by $GABA_A$ and $GABA_B$ receptors (34,56) and by specific taurine receptors (78). Additionally, taurine can also regulate calcium homeostasis in excitable tissues such as the brain and heart (26,41), via an intracellular site of action. Together, the inhibitory and osmoregulatory properties of taurine suggest that it acts as a cytoprotective agent in the brain. Depletion of taurine results in retinal degeneration in cats (70), supporting a role in neuronal survival.

Although most animals possess the ability to synthesize taurine, many are unable to generate sufficient quantities and therefore rely on dietary sources. Taurine transport is thus critical to the maintenance of appropriate levels of taurine in the body. High-affinity, sodium-dependent taurine uptake has been observed in brain and various peripheral tissues (27,64), but little is known about the molecular properties of the taurine transporter(s). Cloning of the taurine transporter will not only help elucidate the function of this important neuro-active molecule, but may also provide important insight into novel therapeutic approaches to treat neurological disorders.

cDNA clones (designated rB14b, rB8b, and rB16a) encoding transporters for two novel GABA transporters and a taurine transporter, respectively, have been isolated from rat brain, and their functional properties have been examined in mammalian cells. The transporters encoded by rB14b and rB8b display high-affinity for GABA (K_m=4μM), and exhibit pharmacological properties distinct from the neuronal GABA transporter; the transporter encoded by rB16a displays high-affinity for taurine. All three are dependent on external sodium and chloride for transport activity. The nucleotide sequences of the three clones predict proteins of 602, 627, and 621 amino acids, respectively. Hydropathy analysis reveals stretches of hydrophobic amino acids suggestive of 12 transmembrane domains, similar to that proposed for other cloned neurotransmitter transporters. The cloning of two additional GABA transporters and a taurine transporter from rat brain reveals previously undescribed heterogeneity in inhibitory amino acid transporter genes.

The use of human gene products in the process of drug development offers significant advantages over those of other species, which may not exhibit the same pharmacological profiles. To facilitate this human-target based approach to drug design in the area of inhibitory amino acid transporters, we used the nucleotide sequences of the rat GAT-2 and GAT-3 cDNAs to clone the human homologue of each gene. cDNA clones (designated hHE7a, hS3a, hFB16a and hFB20a encoding the human homologue of the two novel GABA transporters GAT-2 and GAT-3 have been isolated.

Summary of the Invention

This invention provides an isolated nucleic acid molecule encoding a mammalian GABA transporter. In one embodiment of this invention, the nucleic acid molecule comprises a plasmid designated EVJB-rB14b (ATCC Accession No.). In another embodiment of this invention, the nucleic acid molecule comprises a plasmid designated EVJB-rB8b (ATCC Accession No.).

This invention also provides an isolated nucleic acid molecule encoding a mammalian taurine transporter. In one embodiment of this invention, the nucleic acid molecule comprises a plasmid designated EVJB-rB16a (ATCC Accession No.).

This invention further provides isolated nucleic acid molecules encoding the human homologue of the mammalian GABA transporters. In one embodiment of this invention, the nucleic acid molecule comprises a plasmid designated pcEXV-hGAT-3 (ATCC Accession No.). In another embodiment of this invention, the nucleic acid molecule comprises a plasmid designated pBluescript-hHE7a (ATCC Accession No.). In another embodiment of this invention, the nucleic acid molecule comprises the plasmid pBluescript-hS3a (ATCC Accession No.).

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a mammalian GABA transporter. This invention also provides a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a

sequence included within the sequence of a nucleic acid molecule encoding a mammalian taurine transporter. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human GABA transporter. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human taurine transporter.

This invention further provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a mammalian GABA transporter so as to prevent translation of the mRNA molecule. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a mammalian taurine transporter so as to prevent translation of the mRNA molecule. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a human GABA transporter so as to prevent translation of the mRNA molecule. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a human taurine transporter so as to prevent translation of the mRNA molecule.

A monoclonal antibody directed to a mammalian GABA transporter is provided by this invention. A monoclonal antibody directed to a mammalian taurine transporter is also provided by this invention. A monoclonal antibody

directed to a human GABA transporter is also provided by this invention. A monoclonal antibody directed to a human taurine transporter is also provided by this invention.

This invention provides a pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a mammalian GABA transporter and a pharmaceutically acceptable carrier as well as a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of GABA transporter and a pharmaceutically acceptable carrier.

A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a mammalian taurine transporter and a pharmaceutically acceptable carrier as well as a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a taurine transporter and a pharmaceutically acceptable carrier is also provided by this invention.

A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human GABA transporter and a pharmaceutically acceptable carrier as well as a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a human GABA transporter and a pharmaceutically acceptable carrier is also provided by this invention.

A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human taurine transporter and a pharmaceutically acceptable carrier as well as a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a human taurine transporter and a pharmaceutically acceptable carrier is also provided by this invention.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian GABA transporter so positioned within such genome as to be transcribed into antisense mRNA complementary to mRNA encoding the GABA transporter and when hybridized to mRNA encoding the GABA transporter, the complementary mRNA reduces the translation of the mRNA encoding the GABA transporter.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian taurine transporter so positioned within such genome as to be transcribed into antisense mRNA complementary to mRNA encoding the taurine transporter and when hybridized to mRNA encoding the taurine transporter, the complementary mRNA reduces the translation of the mRNA encoding the taurine transporter.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human GABA transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the human GABA transporter and when hybridized to mRNA encoding the human GABA transporter,

the antisense mRNA thereby reduces the translation of mRNA encoding the human GABA transporter.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human taurine transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the human taurine transporter and when hybridized to mRNA encoding the human taurine transporter, the antisense mRNA thereby reduces the translation of mRNA encoding the human taurine transporter.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian GABA transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the transporter and when hybridized to mRNA encoding the transporter, the antisense mRNA thereby prevents the translation of mRNA encoding the transporter.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian taurine transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the transporter and when hybridized to mRNA encoding the transporter, the antisense mRNA thereby prevents the translation of mRNA encoding the transporter.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human GABA transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to

mRNA encoding the transporter and when hybridized to mRNA encoding the human GABA transporter, the antisense mRNA thereby prevents the translation of mRNA encoding the human GABA transporter.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human taurine transporter so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the human taurine transporter and when hybridized to mRNA encoding the human taurine transporter, the antisense mRNA thereby prevents the translation of mRNA encoding the human taurine transporter.

This invention provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a mammalian GABA transporter on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a mammalian GABA transporter, the protein encoded thereby is expressed on the cell surface, with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a mammalian GABA transporter.

This invention provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a mammalian taurine transporter on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a mammalian taurine transporter, the protein encoded thereby is expressed on the cell surface, with a plurality of drugs, determining those drugs which bind to the mammalian cell,

and thereby identifying drugs which specifically interact with, and bind to, a mammalian taurine transporter.

This invention provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a human GABA transporter on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human GABA transporter, the protein encoded thereby is expressed on the cell surface, with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a human GABA transporter.

This invention provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a human taurine transporter on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a human taurine transporter, the protein encoded thereby is expressed on the cell surface, with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a human taurine transporter.

This invention also provides a method of determining the physiological effects of expressing varying levels of mammalian GABA transporters which comprises producing a transgenic nonhuman animal whose levels of mammalian GABA transporter expression are varied by use of an inducible promoter which regulates mammalian GABA transporter expression.

This invention also provides a method of determining the physiological effects of expressing varying levels of

mammalian taurine transporters which comprises producing a transgenic nonhuman animal whose levels of mammalian taurine transporter expression are varied by use of an inducible promoter which regulates mammalian taurine transporter expression.

This invention also provides a method of determining the physiological effects of expressing varying levels of human GABA transporters which comprises producing a transgenic nonhuman animal whose levels of human GABA transporter expression are varied by use of an inducible promoter which regulates human GABA transporter expression.

This invention also provides a method of determining the physiological effects of expressing varying levels of human taurine transporters which comprises producing a transgenic nonhuman animal whose levels of human taurine transporter expression are varied by use of an inducible promoter which regulates human taurine transporter expression.

This invention further provides a method of determining the physiological effects of expressing varying levels of mammalian GABA transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of mammalian GABA transporter.

This invention further provides a method of determining the physiological effects of expressing varying levels of mammalian taurine transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of mammalian taurine transporter.

This invention further provides a method of determining the physiological effects of expressing varying levels of human GABA transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human GABA transporter.

This invention further provides a method of determining the physiological effects of expressing varying levels of human taurine transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human taurine transporter.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific mammalian GABA transporter allele and a method for diagnosing a predisposition to a disorder associated with the expression of a specific mammalian taurine transporter allele which comprises: a.) obtaining DNA of subjects suffering from the disorder; b.) performing a restriction digest of the DNA with a panel of restriction enzymes; c.) electrophoretically separating the resulting DNA fragments on a sizing gel; d.) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a mammalian GABA or a mammalian taurine transporter and labelled with a detectable marker; e.) detecting labelled bands which have hybridized to the DNA encoding a mammalian GABA or taurine transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f.) preparing DNA obtained for diagnosis by steps a-e; and g.) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether

the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific human GABA transporter allele or a specific human taurine transporter allele which comprises: a.) obtaining DNA of subjects suffering from the disorder; b.) performing a restriction digest of the DNA with a panel of restriction enzymes; c.) electrophoretically separating the resulting DNA fragments on a sizing gel; d.) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human GABA or human taurine transporter and labelled with a detectable marker; e.) detecting labelled bands which have hybridized to the DNA encoding a human GABA or human taurine transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f.) preparing DNA obtained for diagnosis by steps a-e; and g.) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

This invention provides a method for determining whether a substrate not known to be capable of binding to a mammalian transporter can bind to the mammalian GABA transporter which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the GABA transporter with the substrate under conditions permitting binding of substrates known to bind to a

transporter, detecting the presence of any of the substrate bound to the GABA transporter, and thereby determining whether the substrate binds to the GABA transporter.

This invention provides a method for determining whether a substrate not known to be capable of binding to a taurine transporter can bind to a taurine transporter which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the taurine transporter with the substrate under conditions permitting binding of substrates known to bind to a transporter, detecting the presence of any of the substrate bound to the taurine transporter, and thereby determining whether the substrate binds to the taurine transporter.

This invention provides a method for determining whether a substrate not known to be capable of binding to a human GABA transporter can bind to a human GABA transporter which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the human GABA transporter with the substrate under conditions permitting binding of substrates known to bind to a transporter, detecting the presence of any of the substrate bound to the human GABA transporter, and thereby determining whether the substrate binds to the human GABA transporter.

This invention provides a method for determining whether a substrate not known to be capable of binding to a human taurine transporter can bind to a human taurine transporter which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the human taurine transporter with the substrate under conditions permitting binding of substrates known to bind to a transporter, detecting the presence of any of the substrate bound to the

substrate bound to the human taurine transporter, and thereby determining whether the substrate binds to the human taurine transporter.

Brief Description of the Figures

Figure 1. Nucleotide Sequence, Deduced Amino Acid Sequence and Putative Membrane Topology of Two Novel Mammalian GABA Transporters and a Novel Mammalian Taurine Transporter. **A.** Mammalian GABA transporter encoded by GAT-2 (rB14b)(Seq. I.D. Nos. 1 and 2). Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine and ending in the termination codon. Deduced amino acid sequence by translation of a long open reading frame is shown. **B.** Mammalian GABA transporter encoded by GAT-3 (rB8b) (Seq. I.D. Nos. 3, and 4). Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine and ending in the termination codon. Deduced amino acid sequence by translation of a long open reading frame is shown. **C.** Taurine transporter encoded by rB16a (Seq. I.D. Nos. 5 and 6). Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine and ending in the termination codon. Deduced amino acid sequence by translation of a long open reading frame is shown. **D.** Deduced amino acid sequence and putative membrane topology of GABA tranporter GAT-2 (rB14b). Deduced amino acid sequence by translation of a long open reading frame in rB14b is shown. Residues which are identical to those of GAT-3 (rB8b) are shaded. Membrane topology is modeled after that proposed for GAT-1 (21). **E.** Deduced amino acid sequence and putative membrane topology of taurine transporter (rB16a). Deduced amino acid sequence by translation of a long open reading frame in rB16a is shown. Membrane topology is modeled after that proposed for GAT-1 (21).

Figure 2. Alignment of the novel GABA transporters with the rat neuronal GABA transporter, the betaine transporter, and the glycine transporter. The twelve putative α-helical membrane spanning domains (I-XII) are bracketed. Residues identical to those of GAT-2 are shaded. GAT-2 is the GABA transporter encoded by rB14b; GAT-3 is the GABA transporter encoded by rB8b; GAT-1 is the rat neuronal GABA transporter (21), Betaine is the dog betaine transporter (79), and Glycine is the rat glycine transporter (68).

Figure 3. GABA transport by COS cells transfected with clone rB14b and rB8b. Non-transfected COS cells (control) or COS cells transfected with GAT-2 (panel A) or GAT-3 (panel B) were incubated for 10 minutes (37°C) with 50nM [^{3}H]GABA in either HBS (150mM NaCl) or in a similar solution in which Na^+ was replaced by equimolar Li^+ (Na^+-free), or Cl^- was replaced by acetate (in some experiments, calcium gluconate was used instead of calcium acetate; Cl^--free). Data show the specific uptake of GABA, expressed as pmoles/mg protein cellular protein. Data are from a single experiment that was repeated with similar results.

Figure 4. Concentration dependence of GABA transport. COS cells transfected with GAT-2 (panel A) or GAT-3 (panel B) were incubated with the indicated concentrations of [^{3}H]GABA for 30 seconds and the accumulated radioactivity was determined. The specific activity of the [^{3}H]GABA was reduced with unlabeled GABA. Data represent specific transport expressed as nmoles per minute per mg protein, and are from a single experiment that was repeated with similar results (see Text).

Figure 5. Localization of GABA transporters. **A.** Northern blot analysis of mRNAs encoding GAT-2 (rB14b) and GAT-3 (rB8b). Total RNA (25 μg) from rat brain and liver was separated by formaldehyde/agarose gel electrophoresis, blotted to nylon membranes, and hybridized at high stringency with ^{32}P-labeled GABA transporter cDNAs (rB14b and rB8b, respectively). The autoradiogram was developed after a four day exposure. The locations of ribosomal RNAs are indicated at the side. The hybridizing transcripts are ≈2.4kb (GAT-2) and ≈4.7kb (GAT-3). **B.** Tissue distribution of mRNAs encoding GAT-1, GAT-2, and GAT-3 as determined by PCR. Single-stranded cDNA converted from poly A+ RNA was used for PCR amplification (30 cycles) of GABA transporter cDNA sequences. Amplified products were detected by hybridization with specific oligonucleotide probes; autoradiograms of the Southern blots are shown. GAT-1 is the neuronal GABA transporter. GAT-2 is the transporter encoded by rB8b. GAT-3 is the transporter by rB14b. Equivalent samples of poly A+ RNA (not treated with reverse transcriptase) subjected to identical PCR conditions showed no hybridization with the three probes (not shown). Cyclophilin cDNA was amplified to an equal extent from all tissues examined (not shown). Each experiment was repeated at least once with similar results.

Figure 6. Alignment of the taurine transporter with the GABA transporter GAT-1, the betaine transporter, and the glycine transporter. The twelve putative α-helical membrane spanning domains (I-XII) are bracketed. Residues identical to those of the taurine transporter are shaded. Taurine is the taurine transporter encoded by rB16a; GAT-1 is the rat brain GABA transporter (21);

Betaine is the dog betaine transporter (79); Glycine is the rat glycine transporter (68).

Figure 7. Taurine transport by COS cells transfected with clone rB16a. Non-transfected COS cells (control) or COS cells transfected with rB16a were incubated for 10 minutes (37°C) with 50nM [^{3}H]taurine in either HBS (150mM NaCl) or in a similar solution in which Na^+ was replaced by equimolar Li^+ (Na^+-free), or Cl^- was replaced by acetate (Cl^--free). Data show the specific uptake of taurine, expressed as % of control cells. Each bar represents the mean±SEM of 3-7 experiments.

Figure 8. Concentration dependence of taurine transport. COS cells transfected with rB16a were incubated with the indicated concentrations of [^{3}H]taurine for 30 seconds and the accumulated radioactivity was determined. The specific activity of [^{3}H]taurine was reduced with unlabeled taurine. Data represent specific transport expressed as nmoles per minute per mg protein, and are from a single experiment that was repeated with similar results (see Text).

Figure 9. Localization of the taurine transporter. **A.** Tissue distribution of mRNA encoding the taurine transporter as determined by PCR. Single-stranded cDNA converted from poly A+ RNA was used for PCR amplification (30 cycles) of taurine transporter cDNA from a variety of rat tissues. A plasmid containing the cloned taurine transporter was amplified under identical conditions as a control. Amplified products were detected by hybridization with an oligonucleotide probe specific to the taurine transporter; an autoradiogram of the Southern blot is shown. Equivalent samples of poly A+ RNA (not treated with reverse transcriptase) subjected to

identical PCR conditions showed no hybridization with the transporter probe (not shown), indicating that the signals obtained with cDNA were not a result of genomic DNA contamination. The experiment was repeated with similar results. B. Northern blot analysis of mRNA encoding the taurine transporter. Poly A+ RNA (5μg) from a variety of rat tissues was separated by formaldehyde/agarose gel electrophoresis, blotted to a nylon membrane, and hybridized at high stringency with ^{32}P-labeled taurine transporter cDNA (rB16a). The autoradiogram was developed after an overnight exposure. Size standards are indicated at the left in kilobases. The hybridizing transcript is ~6.2 kb.

Figure 10. Nucleotide Sequence and Deduced Amino Acid of Human Transporters. A. Sequence of the Human GAT-2 GABA Transporter. Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the first nucleotide in a partial cDNA clone. Deduced amino acid sequence by translation of a long open reading frame is shown. B. Sequence of the Human GAT-3 GABA Transporter. Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine and ending in the terminating codon. Deduced amino acid sequence by translation of a long open reading frame is shown.

Detailed Description of the Invention

This invention provides an isolated nucleic acid molecule encoding a mammalian GABA transporter. This invention also provides an isolated nucleic acid molecule encoding a mammalian taurine transporter. This invention further provides an isolated nucleic acid molecule encoding a human GABA transporter. As used herein, the term "isolated nucleic acid molecule" means a non-naturally occurring nucleic acid molecule that is, a molecule in a form which does not occur in nature. Examples of such an isolated nucleic acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a mammalian GABA, or mammalian taurine transporter. As used herein, "GABA transporter" means a molecule which, under physiologic conditions, is substantially specific for the neurotransmitter GABA, is saturable, of high affinity for GABA (Km=4μM), and exhibits pharmacological properties distinct from the neuronal GABA transporter. As used herein, "taurine transporter" means a molecule which, under physiologic conditions, is substantially specific for the neurotransmitter taurine, is saturable, and of high affinity for taurine. One embodiment of this invention is an isolated murine nucleic acid molecule encoding a GABA or taurine transporter. Such a molecule may have coding sequences substantially the same as the coding sequences shown in Figure 1A, 1B or 1C. The DNA molecules of Figures 1A (Sequence I.D. No. 1) and 1B (Seq I.D. No.3) encode the sequence of the mammalian GABA transporter genes. The DNA molecule of Figure 1C (Sequence I.D. No. 5) encodes the sequence of a mammalian taurine transporter gene. Another preferred embodiment of this invention is an isolated human nucleic acid molecule

encoding a human GABA transporter. Such a molecule may have coding sequences substantially the same as the coding sequences shown in Figures 10A and 10B. The DNA molecules of Figures 10A (Sequence I.D. No.7) and 10B (Sequence I.D. No.9) encode the sequences of human GABA transporter genes. Another preferred embodiment of this invention is an isolated nucleic acid molecule encoding a human taurine transporter. Such a molecule may have coding sequences substantially similar to the sequence shown in Figure 1C. One means of isolating a mammalian GABA or a mammalian taurine transporter is to probe a mammalian genomic library with a natural or artificially designed DNA probe, using methods well known in the art. In the preferred embodiment of this invention, the mammalian GABA and mammalian taurine transporter are human proteins and the nucleic acid molecules encoding them are isolated from a human cDNA library or a human genomic DNA library. DNA probes derived from the rat GABA transporter genes rB14b and rB8b, and DNA probes derived form the rat taurine transporter gene rB16a are useful probes for this purpose. DNA and cDNA molecules which encode mammalian GABA or mammalian taurine transporters are used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic DNA libraries, by methods described in more detail below. Transcriptional regulatory elements from the 5' untranslated region of the isolated clone, and other stability, processing, transcription, translation, and tissue specificity determining regions from the 3' and 5' untranslated regions of the isolated gene are thereby obtained.

This invention provides a method for obtaining an isolated nucleic acid molecule encoding a human taurine

transporter which comprises using oligonucleotide primers based on the nucleic acid sequence coding for a mammalian taurine receptor and the polymerase chain reaction (PCR) to detect the presence of the nucleic acid molecule coding for the taurine transporter in a human cDNA library. PCR is carried out at reduced annealing temperatures to allow for mismatches between the nucleic acid sequences encoding the rat taurine transporter and nucleic acid sequences encoding the human taurine transporter. Amplified DNA sequences encoding a human taurine transporter are detected by hybridization at reduced hybridization stringency with radiolabelled cDNA encoding the mammalian taurine receptor. A human cDNA library identified by the above method to contain a nucleic acid molecule encoding the human taurine transporter is then screened at low hybridization stringency with the same cDNA probe encoding the mammalian taurine receptor to isolate a cDNA clone encoding a human taurine transporter. A cDNA sequence from the resulting clone can then be used to screen additionally screen a human cDNA or human genomic DNA library to obtain the entire sequence of the human homologue of the mammalian taurine transporter. Primers used in the polymerase chain reaction to initially screen human cDNA libraries to identify human cDNA libraries containing clones encoding a human taurine receptor may be composed of a plurality of degenerate primers based on the sequence of the mammalian taurine transporter. The methods of synthesizing primers, of screening cDNA libraries by PCR to identify libraries containing a cDNA clone encoding the protein of interest are well known by one of skill in the art and examples of this method for obtaining a cDNA clone encoding the human homologue of mammalian transporter are further given below. These same methods can be used to isolate cDNA and genomic DNAs

encoding additional mammalian or human GABA transporter subtypes or taurine transporter subtypes encoded by different genes or encoded by the same gene and generated by alternative splicing of the RNA or rearrangement of the genomic DNA.

This invention provides an isolated nucleic acid molecule which has been so mutated as to be incapable of encoding a molecule having normal transporter activity, and not expressing native transporter. An example of a mutated nucleic acid molecule provided by this invention is an isolated nucleic acid molecule which has an in-frame stop codon inserted into the coding sequence such that the transcribed RNA is not translated into a protein having normal transporter activity.

This invention further provides a cDNA molecule encoding a mammalian GABA transporter, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figure 1A or 1B. (Sequence I.D. Nos. 1 or 3). This invention also provides a cDNA molecule encoding a mammalian taurine transporter, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figure 1C. (Sequence I.D. No. 5). This invention also provides a cDNA molecule encoding a human GABA transporter, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figures 10A (Sequence I.D. No. 7) and 10B (Sequence I.D. No. 9). These molecules and their equivalents were obtained by the means described above.

This invention also provides an isolated protein which is a mammalian GABA transporter. This invention further provides an isolated protein which is a mammalian taurine

transporter. In one embodiment of this invention, the protein is a murine GABA transporter protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figures 1A (Seq. I.D. Nos. 1 and 2) or 1B (Seq. I.D. Nos. 3 and 4). In another embodiment of this invention, the protein is a murine taurine transporter protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figure 1C (Seq. I.D. Nos. 5 and 6). In a preferred embodiment of this invention, the protein is a human GABA transporter protein having an amino acid sequence substantially the same as the sequence shown in Figure 10A (Sequence I.D. Nos. 7 and 8) and Figure 10B (Sequence I.D. Nos. 9 and 10). Another preferred embodiment of this invention, the protein is a human taurine transporter protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figure 1C (Seq. I.D. Nos. 5 and 6). As used herein, the term "isolated protein" is intended to encompass a protein molecule free of other cellular components. One means for obtaining an isolated GABA or taurine transporter is to express DNA encoding the transporter in a suitable host, such as a bacterial, yeast, or mammalian cell, using methods well known to those skilled in the art, and recovering the transporter protein after it has been expressed in such a host, again using methods well known in the art. The transporter may also be isolated from cells which express it, in particular from cells which have been transfected with the expression vectors described below in more detail.

This invention also provides a vector comprising an isolated nucleic acid molecule such as DNA, RNA, or cDNA, encoding a mammalian GABA transporter. This invention also provides a vector comprising an isolated nucleic

acid molecule such as DNA, RNA, or cDNA, encoding a mammalian taurine transporter. This invention also provides a vector comprising an isolated nucleic acid molecule such as DNA, RNA, or cDNA, encoding a human GABA transporter. This invention also provides a vector comprising an isolated nucleic acid molecule such as DNA, RNA, or cDNA, encoding a human taurine transporter. Examples of vectors are viruses such as bacteriophages (such as phage lambda), cosmids, plasmids (such as pUC18, available from Pharmacia, Piscataway, NJ), and other recombination vectors. Nucleic acid molecules are inserted into vector genomes by methods well known to those skilled in the art. Examples of such plasmids are plasmids comprising cDNA having a coding sequence substantially the same as: the coding sequence shown in Figure 1A (Seq. I.D. No. 1) and designated clone pEVJB-rB14b deposited under ATCC Accession No. 75203, the coding sequence shown in Figure 1B (Seq. I.D. No. 3) and designated clone pEVJB-rB8b deposited under ATCC Accession No. 75201, the coding sequence shown in Figure 1C (Seq. I.D. No. 5) and designated pEVJB-rB16a deposited under ATCC Accession No. 75202, the coding sequence shown in Figure 10A, (Sequence I.D. No. 7) designated pBluescript-hHE7a and pBluescript-hS3a and deposited under ATCC Accession Nos. and , respectively, or the coding sequence shown in Figure 10B (SEQ. I.D. No. 9) and designated pcEXV-hGAT-3 and deposited under ATCC Accession No. . Alternatively, to obtain these vectors, insert and vector DNA can both be exposed to a restriction enzyme to create complementary ends on both molecules which base pair with each other and are then ligated together with a ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction site in the vector DNA, which is then

digested with the restriction enzyme which cuts at that site. Other means are also available.

This invention also provides vectors comprising a DNA molecule encoding a mammalian GABA transporter and vectors comprising a DNA molecule encoding a mammalian taurine transporter, adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a mammalian GABA transporter or to the DNA encoding a mammalian taurine transporter as to permit expression thereof. DNA having coding sequences substantially the same as the coding sequence shown in Figure 1A or Figure 1B may usefully be inserted into the vectors to express mammalian GABA transporters. DNA having coding sequences substantially the same as the coding sequence shown in Figure 1C may usefully be inserted into the vectors to express mammalian taurine transporters. This invention also provides vectors comprising a DNA molecule encoding a human GABA transporter adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a human GABA transporter as to permit expression thereof. DNA having coding sequences substantially the same as the coding sequence shown in Figures 10A and 10B may usefully be inserted into the vectors to express human GABA transporters. This invention also provides vectors comprising a DNA molecule encoding a human taurine transporter adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary for expression

of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a human taurine transporter as to permit expression thereof. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Maniatis, et al., Molecular Cloning, Cold Spring Harbor Laboratory, 1982). Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express the transporter. Certain uses for such cells are described in more detail below.

In one embodiment of this invention a plasmid is adapted for expression in a bacterial, yeast, or, in particular, a mammalian cell wherein the plasmid comprises a DNA molecule encoding a mammalian GABA transporter or a DNA molecule encoding a mammalian taurine transporter and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cell so located relative to the DNA encoding a mammalian GABA transporter or to the DNA encoding a mammalian taurine transporter as to permit expression thereof. In another embodiment of this invention a plasmid is adapted for expression in a bacterial, yeast, or, in particular, a mammalian cell wherein the plasmid comprises a DNA molecule encoding a

human GABA transporter or human taurine transporter and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cell so located relative to the DNA encoding a human GABA transporter or human taurine transporter as to permit expression thereof. Suitable plasmids may include, but are not limited to plasmids adapted for expression in a mammalian cell, e.g., EVJB or EXV. Examples of such plasmids adapted for expression in a mammalian cell are plasmids comprising cDNA having coding sequences substantially the same as the coding sequence shown in Figures 1A, 1B, 1C, 10A and 10B and the regulatory elements necessary for expression of the DNA in the mammalian cell. These plasmids have been designated pEVJB-rB14b deposited under ATCC Accession No.75203, pEVJB-rB8b deposited under ATCC Accession No.75201, pEVJB-rB16a deposited under ATCC Accession No.75202, pBluescript-hHE7a and pBluescript-hS3a deposited under ATCC Accession Nos. and , and pcEXV-hGAT-3 deposited under ATCC accession No. , respectively. Those skilled in the art will readily appreciate that numerous plasmids adapted for expression in a mammalian cell which comprise DNA encoding a mammalian GABA transporter, a mammalian taurine transporter, a human GABA transporter or human taurine transporter and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing plasmids and adapted as appropriate to contain the regulatory elements necessary to express the DNA in the mammalian cell. The plasmids may be constructed by the methods described above for expression vectors and vectors in general, and by other methods well known in the art.

The deposits discussed <u>supra</u> were made pursuant to, and in satisfaction of, the provisions of the Budapest Treaty

on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

This invention provides a mammalian cell comprising a DNA molecule encoding a mammalian GABA transporter or a DNA molecule encoding a mammalian taurine transporter, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, which comprises a DNA molecule encoding a mammalian GABA transporter or a DNA encoding a mammalian taurine transporter and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding a mammalian transporter as to permit expression thereof. This invention also provides a mammalian cell comprising a DNA molecule encoding a human GABA transporter or a human taurine transporter, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, which comprises a DNA molecule encoding a human GABA transporter or DNA encoding a human taurine transporter and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding a human transporter as to permit expression thereof. Numerous mammalian cells may be used as hosts, including, but not limited to, the mouse fibroblast cell NIH3T3, CHO cells, HeLa cells, Ltk$^-$ cells, Cos cells, etc. Expression plasmids such as that described <u>supra</u> may be used to transfect mammalian cells by methods well known in the art such as calcium phosphate precipitation, or DNA encoding these transporters may be otherwise introduced into mammalian cells, e.g., by microinjection, to obtain mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding a mammalian GABA transporter,

encoding a mammalian taurine transporter or encoding a human GABA trasnporter.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a mammalian GABA transporter, for example with a coding sequence included within the sequences shown in Figures 1A and 1B. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a taurine transporter, for example with a coding sequence included within the sequence shown in Figure 1C. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human GABA transporter, for example with a coding sequence included within the sequence shown in Figures 10A and 10B. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human taurine transporter, for example with a coding sequence substantially similar to the coding sequence included within the sequence shown in Figure 1C. As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. Nucleic acid probe technology is well known to those skilled in the

art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. Detection of nucleic acid encoding a mammalian GABA transporter, mammalian taurine transporter, human GABA transporter or human taurine transporter is useful as a diagnostic test for any disease process in which levels of expression of the corresponding GABA or taurine transporter are altered. DNA probe molecules are produced by insertion of a DNA molecule which encodes the mammalian GABA transporter, the mammalian taurine transporter, the human GABA transporter or the human taurine transporter or fragments thereof into suitable vectors, such as plasmids or bacteriophages, followed by insertion into suitable bacterial host cells and replication and harvesting of the DNA probes, all using methods well known in the art. For example, the DNA may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the DNA into the vector (discussed above), electrophoresed, and cut out of the resulting gel. Examples of such DNA molecules are shown in Figures 1A, 1B, 1C, 10A and 10B. The probes are useful for 'in situ' hybridization or in order to locate tissues which express this gene family, or for other hybridization assays for the presence of these genes or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA molecule which encodes a mammalian GABA transporter or a mammalian taurine transporter or complementary to the sequence of a DNA molecule which encodes a human GABA transporter or human taurine transporter, are useful as probes for these genes, for their associated mRNA, or for the isolation of related genes by homology screening of

genomic or cDNA libraries, or by the use of amplification techniques such as the Polymerase Chain Reaction.

This invention also provides a method of detecting expression of a GABA transporter on the surface of a cell by detecting the presence of mRNA coding for a GABA transporter. This invention also provides a method of detecting expression of a taurine transporter on the surface of the cell by detecting the presence of mRNA coding for a taurine transporter. This invention further provides a method of detecting the expression of a human GABA or human taurine transporter on the surface of the cell by detecting the presence of mRNA coding for the corresponding GABA or taurine transporter. These methods comprise obtaining total mRNA from the cell using methods well known in the art and contacting the mRNA so obtained with a nucleic acid probe as described hereinabove, under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the transporter by the cell. Hybridization of probes to target nucleic acid molecules such as mRNA molecules employs techniques well known in the art. However, in one embodiment of this invention, nucleic acids are extracted by precipitation from lysed cells and the mRNA is isolated from the extract using a column which binds the poly-A tails of the mRNA molecules (48). The mRNA is then exposed to radioactively labelled probe on a nitrocellulose membrane, and the probe hybridizes to and thereby labels complementary mRNA sequences. Binding may be detected by autoradiography or scintillation counting. However, other methods for performing these steps are well known to those skilled in the art, and the discussion above is merely an example.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a mammalian GABA transporter so as to prevent translation of the mammalian GABA transporter. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a mammalian taurine transporter so as to prevent translation of the mammalian taurine transporter. This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human GABA transporter so as to prevent translation of the human GABA transporter. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human taurine transporter so as to prevent translation of the human taurine transporter. As used herein, the phrase "binding specifically" means the ability of an antisense oligonucleotide to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. The antisense oligonucleotide may have a sequence capable of binding specifically with any sequences of the cDNA molecules whose sequences are shown in Figures 1A, 1B, 1C, 10A and 10B. A particular example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogues of nucleotides.

This invention also provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a mammalian GABA transporter by passing through a cell

membrane and binding specifically with mRNA encoding a mammalian GABA transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. This invention provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a mammalian taurine transporter by passing through a cell membrane and binding specifically with mRNA encoding a mammalian taurine transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. This invention also provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a human GABA transporter by passing through a cell membrane and binding specifically with mRNA encoding a human GABA transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. This invention also provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a human taurine transporter by passing through a cell membrane and binding specifically with mRNA encoding a human taurine transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The oligonucleotide may be coupled to a substance which inactivates mRNA, such as a

ribozyme. The pharmaceutically acceptable hydrophobic carrier capable of passing through cell membranes may also comprise a structure which binds to a transporter specific for a selected cell type and is thereby taken up by cells of the selected cell type. The structure may be part of a protein known to bind a cell-type specific transporter, for example an insulin molecule, which would target pancreatic cells. DNA molecules having coding sequences substantially the same as the coding sequence shown in Figures 1A, 1B, 1C, 10A or 10B may be used as the oligonucleotides of the pharmaceutical composition.

This invention also provides a method of treating abnormalities which are alleviated by reduction of expression of a GABA transporter. This method comprises administering to a subject an effective amount of the pharmaceutical composition described above effective to reduce expression of the GABA transporter by the subject. This invention further provides a method of treating an abnormal condition related to GABA transporter activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to reduce expression of the GABA transporter by the subject. Examples of such abnormal conditions are epilepsy and generalized anxiety. This invention also provides a method of treating abnormalities which are alleviated by reduction of expression of a taurine transporter. This method comprises administering to a subject an effective amount of the pharmaceutical composition described above effective to reduce expression of the taurine transporter by the subject. This invention further provides a method of treating an abnormal condition related to taurine transporter activity which comprises administering to a subject an amount of the pharmaceutical composition described above

effective to reduce expression of the taurine transporter by the subject. Examples of such abnormal conditions are epilepsy, migraine, and ischemia.

Antisense oligonucleotide drugs inhibit translation of mRNA encoding these transporters. Synthetic antisense oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding a GABA transporter or to mRNA encoding a taurine transporter and inhibit translation of mRNA and are useful as drugs to inhibit expression of GABA transporter genes or taurine transporter genes in patients. This invention provides a means to therapeutically alter levels of expression of mammalian GABA or taurine transporters by the use of a synthetic antisense oligonucleotide drug (SAOD) which inhibits translation of mRNA encoding these transporters. Synthetic antisense oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to portions of the nucleotide sequences shown in Figures 1A, 1B, 1C, 10A or 10B of DNA, RNA or of chemically modified, artificial nucleic acids. The SAOD is designed to be stable in the blood stream for administration to patients by injection, or in laboratory cell culture conditions, for administration to cells removed from the patient. The SAOD is designed to be capable of passing through cell membranes in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOD which render it capable of passing through cell membranes (e.g., by designing small, hydrophobic SAOD chemical structures) or by virtue of specific transport systems in the cell which recognize and transport the SAOD into the cell. In addition, the SAOD can be designed for administration only to certain selected cell populations by targeting the SAOD to be recognized by

specific cellular uptake mechanisms which bind and take up the SAOD only within certain selected cell populations. For example, the SAOD may be designed to bind to a transporter found only in a certain cell type, as discussed above. The SAOD is also designed to recognize and selectively bind to the target mRNA sequence, which may correspond to a sequence contained within the sequences shown in Figures 1A, 1B, 1C, 10A or 10B by virtue of complementary base pairing to the mRNA. Finally, the SAOD is designed to inactivate the target mRNA sequence by any of three mechanisms: 1) by binding to the target mRNA and thus inducing degradation of the mRNA by intrinsic cellular mechanisms such as RNAse I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-regulating factors or of ribosomes, or 3) by inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense oligonucleotide drugs have been shown to be capable of the properties described above when directed against mRNA targets (11,76). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for inactivating target mRNA (60). An SAOD serves as an effective therapeutic agent if it is designed to be administered to a patient by injection, or if the patient's target cells are removed, treated with the SAOD in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce transporter expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of GABA or taurine transporters.

This invention provides an antibody directed to the mammalian GABA transporter. This antibody may comprise,

for example, a monoclonal antibody directed to an epitope of a mammalian GABA transporter present on the surface of a cell, the epitope having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian GABA transporter included in the amino acid sequence shown in Figures 1A or 1B. This invention provides an antibody directed to the mammalian taurine transporter. This antibody may comprise, for example, a monoclonal antibody directed to an epitope of a mammalian taurine transporter present on the surface of a cell, the epitope having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian taurine transporter included in the amino acid sequence shown in Figure 1C. This invention provides an antibody directed to a human GABA transporter. This antibody may comprise, for example, a monoclonal antibody directed to an epitope of a human GABA transporter present on the surface of a cell, the epitope having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human GABA transporter included in the amino acid sequence shown in Figures 10A and 10B. This invention provides an antibody directed to a human taurine transporter. This antibody may comprise, for example, a monoclonal antibody directed to an epitope of a human taurine transporter present on the surface of a cell, the epitope having an amino acid sequence substantially similar to the amino acid sequence for a cell surface epitope of the mammalian taurine transporter shown in Figure 1C. Amino acid sequences may be analyzed by methods well known to those skilled in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to form the part of the protein that is inserted

into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figures 1A or 1B will bind to a surface epitope of a mammalian GABA transporter, and antibodies to the hydrophilic amino acid sequences shown in Figure 1C will bind to a surface epitope of a mammalian taurine transporter, as described. Antibodies to the hydrophilic amino acid sequences shown in Figures 10A or 10B will bind to a surface epitope of a human GABA transporter. Antibodies directed to conserved hydrophilic amino acid sequences specific to a mammalian taurine transporter will bind to a surface epitope of a human taurine transporter. Antibodies directed to mammalian or human transporters may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as NIH3T3 cells or Ltk^- cells may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines and the amino acid sequences shown in Figures 1A, 1B, 1C, 10A and 10B. As a still further alternative, DNA, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen. These antibodies are useful to detect the presence of mammalian transporters encoded by the isolated DNA, or to inhibit the function of the transporters in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

This invention also provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of the mammalian transporter, effective to block binding of naturally occurring substrates to the transporter, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a mammalian GABA transporter present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian GABA transporter included in the amino acid sequences shown in Figures 1A and 1B is useful for this purpose. A monoclonal antibody directed to an epitope of a mammalian taurine transporter present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian taurine transporter included in the amino acid sequence shown in Figure 1C is also useful for this purpose.

This invention also provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of the human transporter, effective to block binding of naturally occurring substrates to the transporter, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a human GABA transporter present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human GABA transporter included in the amino acid sequences shown in Figures 10A or 10B is useful for this purpose.

This invention also provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of a human taurine transporter,

effective to block binding of naturally occurring substrates to the human taurine transporter, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to a conserved epitope specific to a mammalian taurine transporter present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian taurine transporter included in the amino acid sequence shown in Figure 1C is useful for this purpose.

This invention also provides a method of treating abnormalities in a subject which are alleviated by reduction of expression of a mammalian transporter which comprises administering to the subject an effective amount of the pharmaceutical composition described above effective to block binding of naturally occurring substrates to the transporter and thereby alleviate abnormalities resulting from overexpression of a mammalian transporter. Binding of the antibody to the transporter prevents the transporter from functioning, thereby neutralizing the effects of overexpression. The monoclonal antibodies described above are both useful for this purpose. This invention additionally provides a method of treating an abnormal condition related to an excess of transporter activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring substrates to the transporter and thereby alleviate the abnormal condition. Some examples of abnormal conditions associated with excess GABA transporter activity are epilepsy and generalized anxiety. Excess taurine transporter activity associated disorders are epilepsy, migraine, and ischemia.

This invention provides methods of detecting the presence of a GABA or a taurine transporter on the surface of a cell which comprises contacting the cell with an antibody directed to the mammalian GABA transporter or an antibody directed to the mammalian taurine transporter, under conditions permitting binding of the antibody to the transporter, detecting the presence of the antibody bound to the cell, and thereby the presence of the mammalian GABA transporter or the presence of the taurine transporter on the surface of the cell. Such methods are useful for determining whether a given cell is defective in expression of GABA transporters or is defective in expression of taurine transporters on the surface of the cell. Bound antibodies are detected by methods well known in the art, for example by binding fluorescent markers to the antibodies and examining the cell sample under a fluorescence microscope to detect fluorescence on a cell indicative of antibody binding. The monoclonal antibodies described above are useful for this purpose.

This invention provides a transgenic nonhuman mammal expressing DNA encoding a mammalian GABA transporter and a transgenic nonhuman mammal expressing DNA encoding a mammalian taurine transporter. This invention further provides a transgenic nonhuman mammal expressing DNA encoding a human GABA transporter and a transgenic nonhuman mammal expressing DNA encoding a human taurine transporter. This invention also provides a transgenic nonhuman mammal expressing DNA encoding a mammalian GABA transporter so mutated as to be incapable of normal transporter activity, and not expressing native GABA transporter and a transgenic nonhuman mammal expressing DNA encoding a mammalian taurine transporter so mutated as to be incapable of normal transporter activity, and not expressing native taurine transporter. This invention

further provides a transgenic nonhuman mammal expressing DNA encoding a human GABA transporter so mutated as to be incapable of normal transporter activity, and not expressing native GABA transporter and a transgenic nonhuman mammal expressing DNA encoding a human taurine transporter so mutated as to be incapable of normal transporter activity, and not expressing native taurine transporter.

This invention provides a transgenic nonhuman mammal whose genome comprises DNA encoding a mammalian GABA transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GABA transporter and which hybridizes to mRNA encoding a GABA transporter thereby reducing its translation and a transgenic nonhuman mammal whose genome comprises DNA encoding a mammalian taurine transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a taurine transporter and which hybridizes to mRNA encoding a taurine transporter thereby reducing its translation. This invention further provides a transgenic nonhuman mammal whose genome comprises DNA encoding a human GABA transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GABA transporter and which hybridizes to mRNA encoding a GABA transporter thereby reducing its translation and a transgenic nonhuman mammal whose genome comprises DNA encoding a human taurine transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a taurine transporter and which hybridizes to mRNA encoding a taurine transporter thereby reducing its translation. The DNA may additionally comprise an inducible promoter or additionally comprise tissue specific regulatory elements, so that expression can be

induced, or restricted to specific cell types. Examples of DNA are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequences shown in Figures 1A, 1B, 1C, 10A and 10B. An example of a transgenic animal is a transgenic mouse. Examples of tissue specificity-determining regions are the metallothionein promotor (46,83) and the L7 promotor (84).

Animal model systems which elucidate the physiological and behavioral roles of mammalian transporters are produced by creating transgenic animals in which the expression of a transporter is either increased or decreased, or the amino acid sequence of the expressed transporter protein is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding a mammalian transporter or homologous animal versions of these genes, by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal (24) or 2) Homologous recombination (7,82) of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these transporters. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native transporter but does express, for example, an inserted mutant transporter, which has replaced the native transporter in the animal's genome by recombination, resulting in underexpression of the transporter. Microinjection adds genes to the genome, but does not remove them, and so is useful for

producing an animal which expresses its own and added transporters, resulting in overexpression of the transporter.

One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium (24). DNA or cDNA encoding a mammalian transporter is purified from a vector (such as plasmids EVJB-rB14b, EVJB-rB8b, or EVJB-rB16a described above) by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

Since the normal action of transporter-specific drugs is to activate or to inhibit the transporter, the transgenic animal model systems described above are useful for

testing the biological activity of drugs directed against these transporters even before such drugs become available. These animal model systems are useful for predicting or evaluating possible therapeutic applications of drugs which activate or inhibit these transporters by inducing or inhibiting expression of the native or trans-gene and thus increasing or decreasing expression of normal or mutant transporters in the living animal. Thus, a model system is produced in which the biological activity of drugs directed against these transporters are evaluated before such drugs become available. The transgenic animals which over or under produce the transporter indicate by their physiological state whether over or under production of the transporter is therapeutically useful. It is therefore useful to evaluate drug action based on the transgenic model system. One use is based on the fact that it is well known in the art that a drug such as an antidepressant acts by blocking neurotransmitter uptake, and thereby increases the amount of neurotransmitter in the synaptic cleft. The physiological result of this action is to stimulate the production of less transporter by the affected cells, leading eventually to underexpression. Therefore, an animal which underexpresses transporter is useful as a test system to investigate whether the actions of such drugs which result in under expression are in fact therapeutic. Another use is that if overexpression is found to lead to abnormalities, then a drug which down-regulates or acts as an antagonist to the transporter is indicated as worth developing, and if a promising therapeutic application is uncovered by these animal model systems, activation or inhibition of the GABA transporter is achieved therapeutically either by producing agonist or antagonist drugs directed against

these GABA transporters or by any method which increases or decreases the expression of these transporters in man.

Further provided by this invention is a method of determining the physiological effects of expressing varying levels of mammalian transporters which comprises producing a transgenic nonhuman animal whose levels of mammalian transporter expression are varied by use of an inducible promoter which regulates mammalian transporter expression. This invention also provides a method of determining the physiological effects of expressing varying levels of mammalian transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of mammalian transporter. Such animals may be produced by introducing different amounts of DNA encoding a mammalian transporter into the oocytes from which the transgenic animals are developed.

This invention provides a method of determining the physiological effects of expressing varying levels of human transporters which comprises producing a transgenic nonhuman animal whose levels of human transporter expression are varied by use of an inducible promoter which regulates transporter expression. This invention also provides a method of determining the physiological effects of expressing varying levels of human transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of the human transporter. Such animals may be produced by introducing different amounts of DNA encoding a human transporter into the oocytes from which the transgenic animals are developed.

This invention also provides a method for identifying a substance capable of alleviating abnormalities resulting

from overexpression of a mammalian transporter comprising administering the substance to a transgenic nonhuman mammal expressing at least one artificially introduced DNA molecule encoding a mammalian transporter and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a mammalian transporter. This invention also provides a method for identifying a substance capable of alleviating abnormalities resulting from overexpression of a human transporter comprising administering the substance to a transgenic nonhuman mammal expressing at least one artificially introduced DNA molecule encoding a human transporter and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a human transporter. As used herein, the term "substance" means a compound or composition which may be natural, synthetic, or a product derived from screening. Examples of DNA molecules are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequences shown in Figures 1A, 1B, 1C, 10A or 10B.

This invention provides a pharmaceutical composition comprising an amount of the substance described <u>supra</u> effective to alleviate the abnormalities resulting from overexpression of GABA transporter and a pharmaceutically acceptable carrier. This invention also provides a pharmaceutical composition comprising an amount of the substance described <u>supra</u> effective to alleviate the abnormalities resulting from overexpression of taurine transporter and a pharmaceutically acceptable carrier. This invention further provides a pharmaceutical composition comprising an amount of the substance

described supra effective to alleviate the abnormalities resulting from overexpression of a human GABA or human taurine transporter and a pharmaceutically acceptable carrier.

This invention also provides a method for treating the abnormalities resulting from overexpression of a mammalian transporter which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from overexpression of a mammalian transporter. This invention further provides a method for treating the abnormalities resulting from overexpression of a human GABA or human taurine transporter which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from overexpression of a human GABA or taurine transporter.

This invention provides a method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a mammalian transporter comprising administering the substance to the transgenic nonhuman mammal described above which expresses only nonfunctional mammalian transporter and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a mammalian transporter. This invention further provides a method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human GABA or human taurine transporter comprising administering the substance to the transgenic nonhuman mammal described above which expresses only nonfunctional human GABA or human taurine transporter and determining

whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a human GABA or human taurine transporter.

This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of transporter and a pharmaceutically acceptable carrier. This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a human GABA or human taurine transporter and a pharmaceutically acceptable carrier.

This invention provides a method for treating the abnormalities resulting from underexpression of a mammalian transporter which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from underexpression of a mammalian transporter. This invention further provides a method for treating the abnormalities resulting from underexpression of a human GABA or human taurine transporter which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from underexpression of a human GABA or human taurine transporter.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific mammalian transporter allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of

the DNA with a panel of restriction enzymes; c) electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a mammalian transporter and labelled with a detectable marker; e) detecting labelled bands which have hybridized to the DNA encoding a mammalian transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f) preparing DNA obtained for diagnosis by steps a-e; and g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific mammalian transporter allele.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific human GABA or human taurine transporter allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human GABA or human taurine transporter and labelled with a detectable marker; e) detecting labelled bands which have hybridized to the DNA encoding a human GABA or human taurine transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects

suffering from the disorder; f) preparing DNA obtained for diagnosis by steps a-e; and g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific human GABA or human taurine transporter allele.

This invention provides a method of preparing the isolated transporter which comprises inducing cells to express transporter, recovering the transporter from the resulting cells, and purifying the transporter so recovered. An example of an isolated GABA transporter is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figures 1A or 1B. An example of an isolated taurine transporter is an isolated protein having substantially the same amino acid sequence shown in Figure 1C. This invention further provides a method for preparing an isolated human GABA transporter which comprises inducing cells to express the human GABA transporter, recovering the human GABA transporter from the resulting cells, and purifying the human GABA transporter so recovered. An example of an isolated human GABA transporter is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figures 10A or 10B. This invention further provides a method for preparing an isolated human taurine transporter which comprises inducing cells to express the human taurine transporter, recovering the human taurine transporter from the resulting cells, and purifying the human taurine transporter so recovered. An example of an isolated

human taurine transporter is an isolated protein having an amino acid sequence substantially similar to the amino acid sequence of a mammalian taurine transporter shown in Figure 1C. For example, cells can be induced to express transporters by exposure to substances such as hormones. The cells can then be homogenized and the transporter isolated from the homogenate using an affinity column comprising, for example, GABA, taurine, or another substance which is known to bind to the transporter. The resulting fractions can then be purified by contacting them with an ion exchange column, and determining which fraction contains transporter activity or binds anti-transporter antibodies.

This invention provides a method of preparing the isolated mammalian GABA transporter which comprises inserting nucleic acid encoding the mammalian GABA transporter in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the transporter produced by the resulting cell, and purifying the transporter so recovered. An example of an isolated GABA transporter is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figures 1A or 1B. This invention also provides a method of preparing the isolated mammalian taurine transporter which comprises inserting nucleic acid encoding a mammalian taurine transporter in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the transporter produced by the resulting cell, and purifying the transporter so recovered. This invention also provides a method of preparing the isolated human GABA transporter which comprises inserting nucleic acid encoding the human GABA transporter in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the human GABA

transporter produced by the resulting cell, and purifying the human GABA transporter so recovered. These methods for preparing GABA or taurine transporters uses recombinant DNA technology methods well known in the art. For example, isolated nucleic acid encoding GABA or taurine transporter is inserted in a suitable vector, such as an expression vector. A suitable host cell, such as a bacterial cell, or a eukaryotic cell such as a yeast cell, is transfected with the vector. GABA or taurine transporter is isolated from the culture medium by affinity purification or by chromatography or by other methods well known in the art.

This invention provides a method for determining whether a substrate not known to be capable of binding to a mammalian GABA transporter can bind to the mammalian GABA transporter which comprises contacting a mammalian cell comprising a DNA molecule encoding a mammalian GABA transporter with the substrate under conditions permitting binding of substrates known to bind to the transporter, detecting the presence of any of the substrate bound to the transporter, and thereby determining whether the substrate binds to the transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequences shown in Figures 1A, or 1B. This invention provides a method for determining whether a substrate not known to be capable of binding to a mammalian taurine transporter can bind to the mammalian GABA transporter which comprises contacting a mammalian cell comprising a DNA molecule encoding a mammalian taurine transporter with the substrate under conditions permitting binding of substrates known to bind to the transporter, detecting the presence of any of the substrate bound to the transporter, and thereby determining whether the

substrate binds to the transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequences shown in Figure 1C.

This invention also provides a method for determining whether a substrate not known to be capable of binding to a human GABA transporter can bind to a human GABA transporter which comprises contacting a mammalian cell comprising a DNA molecule encoding a human GABA transporter with the substrate under conditions permitting binding of substrates known to bind to the transporter, detecting the presence of any of the substrate bound to the transporter, and thereby determining whether the substrate binds to the transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequences shown in Figures 10A or 10B. This invention also provides a method for determining whether a substrate not known to be capable of binding to a human taurine transporter can bind to a human taurine transporter which comprises contacting a mammalian cell comprising a DNA molecule encoding a human taurine transporter with the substrate under conditions permitting binding of substrates known to bind to the transporter, detecting the presence of any of the substrate bound to the transporter, and thereby determining whether the substrate binds to the transporter. Preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is a Cos7 cell. The preferred method for determining whether a substrate is capable of binding to the mammalian transporter comprises contacting a transfected nonneuronal mammalian cell (i.e. a cell that does not naturally express any type of transporter, thus will only express such a transporter if it is transfected into the cell) expressing a transporter

on its surface, or contacting a membrane preparation derived from such a transfected cell, with the substrate under conditions which are known to prevail, and thus to be associated with, in vivo binding of the substrates to a transporter, detecting the presence of any of the substrate being tested bound to the transporter on the surface of the cell, and thereby determining whether the substrate binds to the transporter. This response system is obtained by transfection of isolated DNA into a suitable host cell. Such a host system might be isolated from pre-existing cell lines, or can be generated by inserting appropriate components into existing cell lines. Such a transfection system provides a complete response system for investigation or assay of the functional activity of mammalian transporters with substrates as described above. Transfection systems are useful as living cell cultures for competitive binding assays between known or candidate drugs and substrates which bind to the transporter and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the transporter isolated from transfected cells are also useful for these competitive binding assays. A transfection system constitutes a "drug discovery system" useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic compounds to activate or inhibit the natural functions of the mammalian transporter and/or the human transporter. The transfection system is also useful for determining the affinity and efficacy of known drugs at the mammalian transporter sites and human transporter sites.

This invention provides a method for isolating membranes which comprise GABA or taurine transporters. In a

preferred embodiment of the invention, membranes comprising a GABA or taurine transporter are isolated from transfected cells comprising a plasmid vector which further comprises the regulatory elements necessary for the expression of the DNA encoding a GABA or taurine transporter so located relative to the DNA encoding the GABA or taurine transporter as to permit expression thereof. The DNA may have the coding sequence substantially the same as the sequence shown in Figure 1A, 1B, 1C, 10A or 10B. The host cell may be a bacterial, yeast, or a mammalian cell. Examples of such cells include the mouse fibroblast cell line NIH3T3, CHO cells, HELA cells, Ltk- cells and Y1 cells. A method for isolating membranes which contain a GABA or taurine transporter comprises preparing a cell lysate from cells expressing the GABA or taurine transporter and isolating membranes from the cell lysate. Methods for the isolation of membranes are well known by one of skill in the art. A method for the isolation of membranes from transfected cells is further described by Branchek et al. (1990). Membranes isolated from transfected cells expressing a GABA or taurine transporter are useful for identifying compounds which may include substrates, drugs or other molecules that specifically bind to a GABA or taurine transporter using radioligand binding methods (Branchek et al. 1990) or other methods described herein. The specificity of the binding of the compound to the transporter may be identified by its high affinity for a particular transporter.

This invention further provides a method for the isolation of vesicles from cells expressing a GABA or taurine transporter. In a preferred embodiment of the invention, vesicles comprising a GABA or taurine transporter are isolated from transfected cells

comprising a plasmid vector which further comprises the regulatory elements necessary for the expression of the DNA encoding a GABA or taurine transporter so located relative to the DNA encoding the GABA or taurine transporter as to permit expression thereof. The DNA may have the coding sequence substantially the same as the sequence shown in Figure 1A, 1B, 1C, 10A or 10B. A method for the isolation of vesicles is described by Barber and Jamieson (1970) and by Mabjeesh et al. (1992). Vesicles comprising a GABA or taurine transporter are useful for assaying and identifying compounds, which may include substrates, drugs or other molecules that enhance or decrease GABA or taurine transporter activity. The compounds may modulate transporter activity by interacting directly with the transporter or by interacting with other cellular components that modulate transporter activity. Vesicles provide an advantage over whole cells in that the vesicles permit one to choose the ionic compositions on both sides of the membrane such that transporter activity and its modulation by can be studied under a variety of controlled physiological or non-physiological conditions. Methods for the assay of transporter activity are well known by one of skill in the art and are described herein below and by Kannner (1978) and Rudnick (1977).

This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the mammalian GABA transporter on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a mammalian GABA transporter on the surface of a cell with a plurality of drugs, detecting those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the mammalian GABA

transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequences shown in Figure 1A or 1B. This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the mammalian taurine transporter on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a mammalian taurine transporter on the surface of a cell with a plurality of drugs, detecting those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the mammalian taurine transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequences shown in Figure 1C. This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a human GABA transporter on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a human GABA transporter on the surface of a cell with a plurality of drugs, detecting those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the human GABA transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequences shown in Figures 10A or 10B. This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a human taurine transporter on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding a human taurine transporter on the surface of a cell with a plurality of drugs, detecting those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the human taurine transporter. Various methods of detection may be employed. The drugs may be "labeled"

by association with a detectable marker substance (e.g., radiolabel or a non-isotopic label such as biotin). Preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is a Cos7 cell. Drug candidates are identified by choosing chemical compounds which bind with high affinity to the expressed transporter protein in transfected cells, using radioligand binding methods well known in the art, examples of which are shown in the binding assays described herein. Drug candidates are also screened for selectivity by identifying compounds which bind with high affinity to one particular transporter subtype but do not bind with high affinity to any other transporter subtype or to any other known transporter site. Because selective, high affinity compounds interact primarily with the target transporter site after administration to the patient, the chances of producing a drug with unwanted side effects are minimized by this approach. This invention provides a pharmaceutical composition comprising a drug identified by the method described above and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. Once the candidate drug has been shown to be adequately bio-available following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action for an adequate period to gain the desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models, the drug may be administered to patients by that route of administration determined to make the drug bio-available,

in an appropriate solid or solution formulation, to gain the desired therapeutic benefit.

Applicants have identified individual transporter subtype proteins and have described methods for the identification of pharmacological compounds for therapeutic treatments. Pharmacological compounds which are directed against specific transporter subtypes provide effective new therapies with minimal side effects.

Elucidation of the molecular structures of the neuronal GABA and taurine transporters is an important step in the understanding of GABAergic neurotransmission. This disclosure reports the isolation, amino acid sequence, and functional expression of a cDNA clones from rat brain which encode a GABA transporters and a cDNA clone from rat brain which encodes a taurine transporter. This disclosure reports the isolation, amino acid sequence, and functional expression of cDNA clones which encode human GABA transporters. The identification of these transporters will play a pivotal role in elucidating the molecular mechanisms underlying GABAergic transmission, and should also aid in the development of novel therapeutic agents.

Complementary DNA clones (designated rB14b, rB8b, and rB16a) encoding two GABA transporters and a taurine transporter, respectively, have been isolated from rat brain, and their functional properties have been examined in mammalian cells. The nucleotide sequence of rB14b predicts a protein of 602 amino acids, rB8b predicts a protein of 627 amino acids, and rB16a predicts a protein of 621 amino acids, with 12 highly hydrophobic regions compatible with membrane-spanning domains. When

incubated with 50 nM [^{3}H]GABA, COS cells transiently transfected with rB14b or rB8b accumulated greater than 50-fold as much radioactivity as non-transfected control cells. The transporters encoded by rB14b and rB8b display high-affinity for GABA(Km=4μM) and are dependent on external sodium and chloride. Similarly, when incubated with 50nM [^{3}H]taurine, Cos cells transiently transfected with rB21a accumulated approximately 7-fold as much radioactivity as non-transfected control cells. The pattern of expression of mRNA encoding two GABA transporters has been examined in the rat brain. Additionally, complementary DNA clones (designated hGAT-3, hHE7a, hS3a) and a genomic DNA clone encoding human GABA transporters have been isolated and their functional properties examined in mammalian cells.

Analysis of the GABA and taurine transporter structure and function provides a model for the development of drugs useful for the treatment of epilepsy, generalized anxiety, migraine, ischemia and other neurological disorders.

This invention identifies for the first time three new mammalian transporter proteins, their amino acid sequences, and their mammalian genes. The invention further identifies the human homologues of two mammalian GABA transporter proteins, their amino acid sequence and their human genes. The information and experimental tools provided by this discovery are useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for these new transporter proteins, their associated mRNA molecules or their associated genomic DNAs. The information and experimental tools provided by this discovery will be useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for

these new transporter proteins, their associated mRNA molecules, or their associated genomic DNAs.

Specifically, this invention relates to the first isolation of three mammalian cDNAs and genomic clones encoding GABA and taurine transporters and the first isolation of cDNAs and a genomic clone encoding the human homologues of two mammalian GABA transporters. The new mammalian genes for these transporters identified herein as rB14b, rB8b, and rB16a have been identified and characterized, and a series of related cDNA and genomic clones have been isolated. In addition, the mammalian GABA and mammalian taurine transporters have been expressed in Cos7 cells by transfecting the cells with the plasmids EVJB-rB14b, EVJB-rB8b, and EVJB-rB16a. The pharmacological binding properties of the proteins encoded have been determined, and these binding properties classify these proteins as GABA transporters and a taurine transporter. Mammalian cell lines expressing the mammalian and human GABA transporters and the mammalian taurine transporter on the cell surface have been constructed, thus establishing the first well-defined, cultured cell lines with which to study the GABA and taurine transporters.

This invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative, and are not meant to limit the invention as described herein, which is defined by the claims which follow thereafter.

MATERIALS and METHODS

Materials for Mammalian GABA Transporter Studies: $[^3H]GABA^3$ (98.9Ci/mmole) was obtained from New England Nuclear (Boston, MA). β-alanine, betaine and L-DABA (L-(2,4) diaminobutyric acid) were from Sigma Chemical Company (St. Louis, MO); guvacine, nipecotic acid, OH-nipecotic (hydroxynipecotic acid), and THPO (4,5,6,7-tetrahydroisoxazolo (4,5-c]pyridin-3-ol) were from RBI (Natick, MA). ACHC (cis-3-aminocyclohexanecarboxylic acid) was kindly provided by Drs. Richard Milius and William White of the NIMH Chemical Synthesis Program.

Materials for Mammalian Taurine Transporter Studies: $[^3H]$taurine (25.6Ci/mmole) was from New England Nuclear (Boston, MA); taurine, $GABA^2$, hypotaurine, AEPA, AMSA, APSA, CSA, MEA, and β-alanine were from Sigma Chemical Corporation (St. Louis, MO); GES was a kind gift of Dr. J. Barry Lombardini (Department of Pharmacology, Texas Tech University).

Cloning and Sequencing of Mammalian GABA Transporters: A rat brain cDNA library in the Lambda ZAP II vector (Stratagene, La Jolla, CA) was screened at reduced stringency using probes representing the complete coding region of the rat GABA transporter cDNA (GAT-1 (21)). Exact primers derived from the nucleotide sequence of GAT-1 were used to generate GAT-1 PCR products from randomly-primed rat brain cDNA; the GAT-1 probes were then labeled and used to screen the library under reduced stringency as previously described (68). Lambda phage hybridizing with the probes at low stringency were plaque purified and rescreened at high stringency to eliminate clones which were identical to GAT-1. One of the clones hybridizing at high stringency was subsequently confirmed by sequence analysis to encode GAT-1 (21). Clones hybridizing only at low stringency were converted to

phagemids by in vivo excision with f1 helper phage. Nucleotide sequences of double-stranded cDNAs in pBluescript were analyzed by the Sanger dideoxy nucleotide chain-termination method (59) using Sequence (U.S. Biochemical Corp., Cleveland, Ohio).

Expression of Mammalian GABA Transporters: cDNA clones (designated rB14b and rB8b) representing the complete coding regions of two putative transporters were cloned into the eukaryotic expression vector pEVJB (modified from pcEXV-3; (51)). Utilizing restriction enzyme sites present in pBluescript, rB14b was subcloned as a 2.0 kb HindIII/XbaI fragment which contained 126 base pairs of 5'-untranslated sequence and 94 base pairs of 3'-untranslated sequence. Similarly, rB8b was subcloned as a 2.1 kb XbaI/SalI fragment containing 0.3 kb of 3'-untranslated sequence. Transient transfections of COS cells were carried out using DEAE-dextran with DMSO according to the method of Lopata et al. (44) with minor modifications. COS cells were grown (37°C., 5%CO_2) in high glucose Dulbecco's modified Eagle medium supplemented with 10% bovine calf serum, 100 U/ml penicillin G, and 100 μg/ml streptomycin sulfate. Cells were routinely used two days after transfection for transport studies.

Transport Studies of Mammalian GABA Transporters: To measure transport, COS cells grown in 6-well (well diameter = 35mm) or 24-well (well diameter = 18mm) plates were washed 3X with HEPES-buffered saline (HBS, in mM: NaCl, 150; HEPES, 20; $CaCl_2$, 1; glucose, 10; KCl, 5; $MgCl_2$, 1; pH 7.4) and allowed to equilibrate in a 37°C water bath. After 10 minutes the medium was removed and a solution containing [^{3}H]GABA (New England Nuclear, sp. activity = 89.8Ci/mmole) and required drugs in HBS was

added (1.5 ml/35mm well; 0.5ml/18mm well). Non-specific uptake was defined in parallel wells with 1mM unlabeled substrate, and was subtracted from total uptake (no competitor) to yield specific uptake; all data represent specific uptake. Plates were incubated at 37°C for 10 minutes unless indicated otherwise, then washed rapidly 3x with ice-cold HBS. Cells were solubilized with 0.05% sodium deoxycholate/0.1N NaOH, an aliquot neutralized with 1N HCl, and radioactivity was determined by scintillation counting. Protein was quantified in an aliquot of the solubilized cells using a BIO-RAD protein assay kit, according to the manufacturers directions.

Northern Blot Analysis of RNA Encoding Mammalian Transporters:

Total cellular RNA was isolated from rat brain and liver using RNazol (Cinna/Biotecx Laboratories Inc.; Houston, TX) as outlined by the manufacturer. Denatured RNA samples (25μg) were separated in a 1.0% agarose gel containing 3.3% formaldehyde. RNAs were transferred to nylon membranes (Genescreen Plus; New England Nuclear, Boston, MA) by overnight capillary blotting in 10X SSC. Northern blots were rinsed and then baked for 2 hours at 80°C under vacuum. Prehybridization was for 2 hours at 65°C in a solution containing 50% formamide, 1M NaCl, 10% dextran sulfate, and 1% sodium dodecyl sulfate. Blots were hybridized overnight at 65°C with ^{32}P-labeled DNA probes (randomly primed GAT-2 or GAT-3 full-length cDNA clones) in prehybridization mixture containing 100 μg/ml sonicated salmon sperm DNA. The blots were washed successively in 2X SSC/2% SDS, 1X SSC/2% SDS, and 0.2X SSC/2% SDS at 65°C, then exposed to Kodak XAR-5 film with one intensifying screen at -90°C for four days.

Tissue Localization Studies: To identify tissues expressing mRNAs for the novel GABA transporters and the previously cloned GABA transporter GAT-1 (21), specific PCR primers (25mers) were designed such that ≈700 base pair fragments encoding TMs 1 through 5 of each transporter could be amplified and detected by hybridization with ^{32}P-labeled oligonucleotides. For rB14b, the sequences of the sense and anti-sense oligonucleotides were derived from amino acids 36 to 43 (5'-GACCAACAAGATGGAGTTCGTACTG) and 247 to 254 (5'-TGTTACTCCTCGGATCAACAGGACC); for rB8b, the oligonucleotides were derived from amino acids 52 to 60 (5'-GGAGTTCGTGTTGAGCGTAGGAGAG) and 271 to 279 (5'-GAACTTGATGCCTTCCGAGGCACCC); and for GAT-1 (21), the oligonucleotide sequences were derived from amino acids 50 to 57 (5'-ACGCTTCGACTTCCTCATGTCCTGT) and 274 to 282 (5'-GAATCAGACAGCTTTCGGAAGTTGG). Primers were also designed to amplify the cDNA encoding cyclophilin, a constitutively expressed gene, as a control (5'-GTCTGCTTCGAGCTGTTTGCAGACA, sense; 5'-TTAGAGTTGTCCACAGTCGGAGATG, anti-sense) (12). To detect amplified sequences, oligonucleotide probes were synthesized for GAT-1, rB14b, and rB8b which corresponded to amino acids 196 to 219, 161 to 183, and 207 to 229, respectively. Each probe was shown to hybridize with its respective transporter cDNA and not with any other transporter cDNA under study.

Poly A+ RNA (1 μg, Clonetech, Palo Alto, CA) from each of seven rat tissues was converted to single-stranded cDNA by random priming using Superscript reverse transcriptase (BRL, Gaithersburg, MD). PCR reactions were carried out in a buffer containing 20mM Tris (pH 8.3), 50 mM KCl, 1.5mM $MgCl_2$, 0.001% gelatin, 2mM dNTP's, 1μM each primer, and Taq polymerase with either cDNA, RNA, water, or a

control plasmid for 30 cycles of 94°C./2 min., 68°C./2 min., 72°C./3 min. PCR products were separated by electrophoresis in 1.2% agarose gels, blotted to nylon membranes (Genescreen Plus; New England Nuclear, Boston, MA), and hybridized at 40°C. overnight with ^{32}P-labeled oligonucleotide probes in a solution containing 50% formamide, 10% dextran sulfate, 5X SSC, 1X Denhardt's, and 100 μg/ml sonicated salmon sperm DNA. Blots were washed successively in 2 X SSC at room temperature and 0.1 X SSC at 50°C., and exposed to Kodak XAR film for 0.5 to 4 hours with an intensifying screen at -70°C.

Cloning and Sequencing of Mammalian Taurine Receptor: A rat brain cDNA library in the Lambda ZAP II vector (Stratagene, La Jolla, CA) was screened at low stringency with the complete coding region of the rat GABA transporter cDNA (GAT-1; (21)). Exact primers were used to generate PCR products from randomly-primed rat brain cDNA; the products were labeled and used to screen the library under reduced stringency (25% formamide, 40°C. hybridization; 0.1X SSC, 40°C. wash) as previously described (68). Lambda phage hybridizing at low stringency with the GAT-1 sequence were plaque purified and rescreened with the same probes at high stringency (50% formamide, 40°C. hybridization; 0.1X SSC, 50°C. wash) to eliminate clones identical to GAT-1. Clones hybridizing only at low stringency were converted to phagemids by in vivo excision with f1 helper phage. Nucleotide sequences of double-stranded cDNAs in pBluescript were analyzed by the Sanger dideoxy nucleotide chain-termination method (59) using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio).

Expression of Mammalian Taurine Transporter: A complementary DNA (designated rB16a) containing the

complete coding region of a putative transporter was cloned into the eukaryotic expression vector pEVJB (modified from pcEXV-3; (51)) as a 2.5 kb XbaI\SalI fragment using restriction enzyme sites within the vector. In addition to the coding region, 0.1 kb of 5'-untranslated sequence and 0.5 kb of 3'-untranslated sequence were included in the construct. Transient transfections of COS cells with the plasmid pEVJB-rB16a were carried out using DEAE-dextran with DMSO according to the method of Lopata et al. (44) with minor modifications. COS cells were grown (37°C., 5% CO_2) in high glucose Dulbecco's modified Eagle medium supplemented with 10% bovine calf serum, 100 U/ml penicillin G, and 100 µg/ml streptomycin sulfate. Cells were routinely used two days after transfection for transport studies.

Transport Studies of Mammalian Taurine Transporter: To measure transport, COS cells grown in 6-well (well diameter = 35mm) or 24-well (well diameter = 18mm) plates were washed 3X with HEPES-buffered saline (HBS, in mM: NaCl, 150; HEPES, 20; $CaCl_2$, 1; glucose, 10; KCl, 5; $MgCl_2$, 1; pH 7.4) and allowed to equilibrate in a 37°C water bath. After 10 minutes the medium was removed and a solution containing [^{3}H]taurine (New England Nuclear, sp. activity = 25.6 Ci/mmole) and required drugs in HBS was added (1.5 ml/35mm well; 0.5ml/18mm well). Non-specific uptake was defined in parallel wells with 1mM unlabeled taurine and was subtracted from total uptake (no competitor) to yield specific uptake; all data represent specific uptake. Plates were incubated at 37°C for 10 minutes unless indicated otherwise, then washed rapidly 3X with ice-cold HBS. Cells were solubilized with 0.05% sodium deoxycholate/0.1N NaOH), an aliquot was neutralized with 1N HCl, and radioactivity was determined by scintillation counting. Protein was quantified in an

aliquot of the solubilized cells using a BIO-RAD protein assay kit, according to the manufacturer's directions.

PCR Tissue Localization Studies of Mammalian Taurine Transporter: To identify tissues expressing mRNA for the taurine transporter, exact primers (25mers) were designed such that a 707 base pair fragment of rB16a could be amplified from cDNA and detected by Southern blot analysis. The sequences of the sense and anti-sense primers were derived from amino acids 40 to 47 (5'-TCAGAGGGAGAAGTGGTCCAGCAAG) and 268 to 275 (5'-ATTTCATGCCTTCACCAGCACCTGG), respectively. Primers were also designed to amplify the cDNA encoding cyclophilin (12), a constitutively expressed gene, as control (5'-ACGCTTCGACTTCCTCATGTCCTGT, sense; 5'-TTAGAGTTGTCCACAGTCGGAGATG, antisense). To detect amplified sequences, an oligonucleotide probe was synthesized (corresponding to amino acids 249 to 271) which was specific for rB16a. Poly A+ RNA (1 μg, Clontech, Palo Alto, CA) from each of seven rat tissues was converted to single-stranded cDNA by random priming using Superscript reverse transcriptase (BRL, Gaithersburg, MD). PCR reactions were carried out in a buffer containing 20mM Tris (pH 8.3), 50 mM KCl, 1.5mM $MgCl_2$, 0.001% gelatin, 2mM dNTP's, 1μM each primer, Taq polymerase, and either cDNA, RNA, water, or a control plasmid containing rB16a for 30 cycles of 94°C./2 min., 68°C./2 min., 72°C./3 min. PCR products were separated by electrophoresis in 1.2% agarose gels, blotted to nylon membranes (Genescreen Plus; New England Nuclear, Boston, MA), and hybridized at 40°C. overnight with specific ^{32}P-labeled oligonucleotides in a solution containing 50% formamide, 10% dextran sulfate, 5X SSC, 1X Denhardt's, and 100 μg/ml of sonicated salmon sperm DNA. Blots were washed at high-stringency (0.1X SSC, 50°C.) and exposed

to Kodak XAR film for 0.5 to 4 hours with one intensifying screen at -70°C.

Northern Blot Analysis of mRNA encoding Mammalian Taurine Transporter: Samples of poly A^+ RNA isolated from each of eight rat tissues (5 μg, Clontech; Palo Alto, CA) were separated in a 1.0% agarose gel containing 3.3% formaldehyde and transferred to a nylon membrane (Genescreen Plus; New England Nuclear, Boston, MA) by overnight capillary blotting in 10X SSC. Prior to hybridization, the Northern blot was incubated for 2 hours at 42°C. in a solution containing 50% formamide, 1M NaCl, 10% dextran sulfate, and 1% sodium dodecyl sulfate (SDS). The blot was hybridized overnight at 42°C. with ^{32}P-labeled DNA probe (randomly-primed HindIII/KpnI fragment of rB16a representing amino acids 6-336) in the prehybridization solution containing 100 μg/ml sonicated salmon sperm DNA. The blot was washed successively in 2X SSC/2% SDS, 1X SSC/2% SDS, and 0.2X SSC/2% SDS at 65°C. and exposed to Kodak XAR-5 film with one intensifying screen at -70°C. for 1-4 days. To confirm that equal amounts of RNA were present in each lane, the same blot was rehybridized with a probe encoding cyclophilin (12).

Use of PCR to Identify human cDNA Libraries for Screening: For hGAT-2, the sequences of the rat PCR primers were 5'-GACCAACAAGATGGAGTT (sense) and 5'-TGTTACTCCTCGGATCAA (antisense). PCR reactions were carried out in a buffer containing 20mM Tris (pH 8.3), 50 mM KCl, 1.5mM $MgCl_2$, 0.001% gelatin, 2mM dNTP's, 1μM each primer, Taq polymerase, and an aliquot of a lambda phage library, water, or a control plasmid for 40 cycles of 94°C. for 2 min., 50°C. for 2 min., and 72°C. for 3 min. For hGAT-3, the sequences of the degenerate primers were 5'-TGGAATTCG(G/C)CAA(C/T)GTITGG(C/A)GITT(C/T)CCITA

(sense) and 5'-TCGCGGCCGCAA(A/G)AAGATCTGIGTIGCIGC(A/G)TC (antisense). PCR reactions were carried out as described above for 40 cycles of 94°C. for 2 min., 40°C. for 2 min., and 72°C. for 3 min. PCR products were separated by electrophoresis in 1.2% agarose gels, blotted to nylon membranes (Genescreen Plus; New England Nuclear, Boston, MA), and hybridized at 40°C. overnight with ^{32}P-labeled probes in a solution containing 25% formamide, 10% dextran sulfate, 5X SSC, 1X Denhardt's, and 100 µg/ml of sonicated salmon sperm DNA. Blots were washed at low stringency (0.1X SSC, 40°C.) and exposed to Kodak XAR film for up to three days with one intensifying screen at -70°C.

Isolation and Sequencing of Human Clones: Human cDNA libraries in the Lambda ZAP II vector (Stratagene, La Jolla, CA) that were identified as containing hGAT-2 or hGAT-3 were screened under either reduced stringency (25% formamide, 40°C. hybridization; 0.1X SSC, 40°C. wash) or high stringency (50% formamide, 40°C. hybridization; 0.1X SSC, 50°C. wash). Hybridizing lambda phage were plaque purified and converted to phagemids by in vivo excision with f1 helper phage. Nucleotide sequences of double-stranded cDNAs in pBluescript were analyzed by the Sanger dideoxy nucleotide chain-termination method (59) using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio). Fragments of genomic clones in the lambda FIX II vector were subcloned into pUC18 prior to double-stranded sequencing.

Preparation of Primary Brain Cell Cultures: Astrocytes, neurons and meningeal fibroblasts were prepared from the brains of E19 embryonic rats. Briefly, the brains were removed, dissected free of meninges, and trypsinized. Cells were dissociated mechanically by passage through a

Pasteur pipet, and resuspended in DMEM containing 10% fetal bovine serum and antibiotics. The cells were added to tissue culture dishes that had been previously coated with 10μM poly-D-lysine.

For astrocytes, the cells were plated at a density of approximately $3x10^6$ cells per 100mm dish. The astrocytes were allowed to reach confluence, then passaged 1 or 2 times prior to harvesting. For neurons, a plating density of $15x10^6$ cells per 100mm dish was employed; the medium was supplemented with insulin. Cytosine arabinoside (ara-C) was added to a final concentration of 10μM on day 2 or 3 to inhibit the proliferation of non-neuronal cells. The neurons were harvested 1 week after plating. To obtain meningeal fibroblasts the meninges were trypsinized, then mechanically dissociated as described above. The cells recovered from a single embryo were plated into a 100mm dish, grown to confluence, and passaged 1-2 times prior to harvesting.

Isolation of RNA from Cell Cultures: Plates were placed on ice and quickly rinsed twice with ice-cold phosphate-buffered saline (PBS). Cells were then dissolved in 10mls lysis solution (7M urea, 350mM NaCl, 2% sodium dodecyl sulfate (SDS), 1mM EDTA, and 10 mM Tris-HCl, pH 8.0) and transferred to a sterile tube. Lysates were homogenized (Virtis, lowest speed, 5 seconds) and then digested with proteinase K (0.1mg/ml) at 37°C. for 30 minutes. Samples were extracted twice with phenol/chloroform and once with chloroform before ethanol precipitation. Total RNA was collected by centrifugation, resuspended in diethylpyrocarbonate (DEPC)-treated water, and stored at -20°C. until use.

Detection of Transporter mRNAs using PCR: To identify cell types expressing mRNAs for the GABA transporters GAT-1, GAT-2, and GAT-3, specific PCR primers (25mers) were designed such that ≈700 base pair fragments encoding transmembrane domains 1 through 5 of each transporter could be amplified and detected by hybridization with ^{32}P-labeled oligonucleotides. For rB14b (GAT-2), the sequences of the sense and anti-sense oligonucleotides were derived from amino acids 36 to 43 (5'-GACCAACAAGATGGAGTTCGTACTG) and 247 to 254 (5'-TGTTACTCCTCGGATCAACAGGACC); for rB8b (GAT-3), the oligonucleotides were derived from amino acids 52 to 60 (5'-GGAGTTCGTGTTGAGCGTAGGAGAG) and 271 to 279 (5'-GAACTTGATGCCTTCCGAGGCACCC); and for GAT-1 (21), the oligonucleotide sequences were derived from amino acids 50 to 57 (5'-ACGCTTCGACTTCCTCATGTCCTGT) and 274 to 282 (5'-GAATCAGACAGCTTTCGGAAGTTGG). To detect amplified sequences, oligonucleotide probes were synthesized for GAT-1, GAT-2, and GAT-3 which corresponded to amino acids 196 to 219, 161 to 183, and 207 to 229, respectively. Each probe was shown to hybridize with its respective transporter cDNA and not with the other transporter cDNAs.

Total RNA (0.5μg) isolated from cultured neurons, astrocytes, and fibroblasts was converted to single-stranded cDNA by random priming using Superscript reverse transcriptase (BRL, Gaithersburg, MD). PCR reactions were carried out in a buffer containing 20mM Tris (pH 8.3), 50 mM KCl, 1.5mM $MgCl_2$, 0.001% gelatin, 2mM dNTP's, 1μM each primer, and Taq polymerase with either cDNA, RNA, water, or a control plasmid for 30 cycles of 94°C./2 min., 68°C./2 min., 72°C./3 min. PCR products were separated by electrophoresis in 1.2% agarose gels, blotted to nylon membranes (Genescreen Plus; New England

Nuclear, Boston, MA), and hybridized at 40°C. overnight with ^{32}P-labeled oligonucleotide probes in a solution containing 50% formamide, 10% dextran sulfate, 5X SSC, 1X Denhardt's, and 100 μg/ml sonicated salmon sperm DNA. Blots were washed successively in 2X SSC, 0.1% SDS at room temperature and 0.1X SSC, 0.1% SDS at 50°C., and exposed to Kodak XAR film for 0.5 to 4 hours with an intensifying screen at -70°C.

In Situ Hybridization: Male Sprague-Dawley rats (Charles River) were decapitated and the brains rapidly frozen in isopentane. Sections were cut on a cryostat, thaw-mounted onto poly-L-lysine coated coverslips, and stored at -80°C until use. Tissue was fixed in 4% paraformaldehyde, treated with 5mM dithiothreitol (DTT), acetylated (0.25% acetic anhydride in 0.1M triethanolamine), and dehydrated. Tissue was prehybridized (1 hour, 40°C) in a solution containing 50% formamide, 4X SSC (0.6M NaCl/0.06M sodium citrate), 1X Denhardt's solution (0.2% polyvinylpyrrolidine, 0.2% Ficoll, 0.2% bovine serum albumin), 50mM DTT, 500μg/ml salmon sperm DNA, 500μg/ml yeast tRNA, 10% dextran sulfate, then hybridized overnight with ^{35}S-labeled anti-sense oligonucleotides (45mers) in the same solution. After washing and dehydration, sections were apposed to Kodak X-OMAT AR film for 4 days at -20°C. To verify the specificity of the hybridization signal, parallel tissues were pretreated with 100 μg/ml RNase A (37°, 30 minutes) prior to hybridization. Two different oligonucleotides designed to separate regions of the GABA transporters (loop region between transmembrane domains III and IV, 3'untranslated region) showed identical patterns of hybridization.

1. GABA Transporters

RESULTS

Cloning of New Mammalian GABA Transporter Sequences:

We screened a rat brain cDNA library at low stringency with probes encoding the rat neuronal GABA transporter (GAT-1; (21)) in order to identify additional inhibitory amino acid transporter genes. Two clones were identified which hybridized at low but not at high stringency with the GABA transporter probes. DNA sequence analysis revealed that the clones encoded putative transporters which were structurally related to GAT-1. The first clone, rB14b, contained a 2.0 kb sequence with an open reading frame of 1806 base pairs which could encode a protein of 602 amino acids (Figure 1A). The second clone, rB8b, contained a 2.1 kb sequence which had an open reading frame of 1881 base pairs encoding a protein of 627 amino acids (Figure 1B). rB14b and rB8b exhibited 59% nucleotide identity throughout the coding region with the neuronal rat GABA transporter (GAT-1) and 70% nucleotide identity with each other. Comparison to sequences in Genbank and EMBL data bases demonstrated that both nucleotide sequences were novel and that the most homologous sequence was the rat GABA transporter GAT-1 (21). Subsequent comparisons which included recently cloned transporters revealed that the most closely related sequence is the canine betaine transporter (79) which exhibits 69% nucleotide identity with both rB14b and rB8b. The taurine transporter (66) and the glycine transporter (68) are also significantly related, exhibiting ~64% and ~56% nucleotide identity, respectively, to both rB14b and rB8b.

The amino acid sequence deduced from the nucleotide sequence of rB14b is shown in Figure 1D modeled after the

proposed membrane topology of GAT-1 (21). Residues identical to those in rB8b are shaded and represent 67% amino acid identity between the two clones. The translation products of both rB14b and rB8b are predicted to have relative molecular masses of ≈68,000 Daltons. Hydropathy analyses indicate the presence of 12 hydrophobic domains in both proteins which may represent membrane spanning segments. For each transporter, several potential sites for Asn-linked glycosylation are found in the extracellular loop between the third and fourth transmembrane domains. Comparison and alignment of the deduced amino acid sequences of rB14b (GAT-2) and rB8b (GAT-3) with the neuronal GABA transporter (GAT-1) (Figure 2) revealed 52.5% and 52% amino acid identities, respectively. The betaine transporter (Figure 2), which can also transport GABA (79) exhibited a significantly higher degree of homology-- 68% and 65% amino acid identities to rB14b and rB8b, respectively. Similarly, the transporter for taurine (66) , an inhibitory amino acid, is 61% homologous to both. In contrast, comparison of the new transporters with the rat glycine transporter (Figure 2 and Ref.(68)) or the human norepinephrine transporter (55) showed a lower degree of amino acid identity (43-45%), similar to that between the neuronal GABA and norepinephrine transporters (46%). These data suggested that the new sequences might encode additional amino acid transporters expressed in the brain. To explore this possibility, the sequences were each placed in a mammalian expression vector, transfected into COS cells, and screened for transport of a variety of radioloabeled neurotransmitters and amino acids. These studies revealed (see below) that rB14b and rB8b encode novel GABA transporters with pharmacological properties distinct from the neuronal GABA transporter.

Pharmacological Characterization of Mammalian GABA Transporters:

COS cells transiently transfected with rB14b or rB8b (COS/rB14b and COS/rB8B, respectively) accumulated more [^{3}H]GABA than non-transfected control cells; representative experiments are shown in Figure 3. During a 10 minute incubation (37°C) with a low concentration of [^{3}H]GABA, specific uptake was increased 52±11-fold (mean±SEM, n=6) and 64±12-fold (n=5) over control for rB14b and rB8b, respectively. In contrast, the uptake of [^{3}H]glutamate, [^{3}H]glycine, [^{3}H]5-HT, [^{3}H]dopamine, and [^{3}H]taurine was unaltered. Specific uptake represented greater than 95% of total uptake in transfected cells. Uptake of [^{3}H]GABA was not observed following mock transfection or transfection with an irrelevant insert, indicating that the enhanced uptake was not the result of non-specific perturbation of the membrane. The transport of [^{3}H]GABA by both COS/rB14b and COS/rB8b was decreased >95% when Na^+ was replaced by Li^+ (Table 1); similar results were obtained with COS cells expressing GAT-1 (COS/GAT-1), which we re-cloned (see Materials and Methods). When Cl^- was replaced by acetate, [^{3}H]GABA transport by COS/GAT-1 was nearly completely eliminated (Table 1), consistent with previous results obtained with this transporter (21,29). In contrast, transport by COS/rB14b and COS/rB8b was decreased to 43 and 20% of control, respectively (Table 1). The difference in sensitivity to removal of chloride exhibited by the three transporters was statistically significant (GAT-1 vs. COS/rB14b, $p<0.001$; GAT-1 vs. rB8b, $p<0.05$; rB14b vs. rB8b, $p<0.05$).

To determine the affinity of GABA for the cloned transporters, COS/rB14b and COS/rB8b were incubated with various concentrations of [^{3}H]GABA and the specific

accumulation of radioactivity was determined. Accumulation of [^{3}H]GABA was dose-dependent and reached saturation at higher concentrations (Figure 4). Non-linear regression analysis of the data yielded the following values: K_M = 8±3μM and 12±6μM, and V_{MAX}= 2.5±1.2 and 3.0±0.9 nmoles/mg protein for COS/rB14b and COS/rB8b, respectively (mean ± SEM, n=4 experiments). Taken together, these data indicate that both rB14b and rB8b encode saturable, high-affinity, sodium- and chloride-dependent GABA transporters. Accordingly, we propose the terms GAT-2 and GAT-3 for the transporters encoded by rB14b and rB8b, respectively, according to the nomenclature proposed by Guastella et al. (21).

To determine the pharmacological properties of the cloned GABA transporters, we examined the ability of various drugs to inhibit the accumulation of [^{3}H]GABA by GAT-2 and GAT-3; for comparison, we also examined the pharmacology of GAT-1. As shown in Table 2, the pharmacological properties of GAT-2 and GAT-3 are similar to one another, but differ considerably from GAT-1. For example, β-alanine, a ligand reported to be selective for glial GABA transport (36), is more potent at the new cloned transporters than at GAT-1. In contrast, ACHC, guvacine, nipecotic acid, and hydroxynipecotic acid are more potent at GAT-1 than at GAT-2 and GAT-3. Interestingly, the two newly cloned tranporters can be distinguished by L-DABA which displays high affinity for GAT-2 as well as GAT-1, but is less potent at GAT-3.

To further chararacterize the pharmacological properties of GAT-2 and GAT-3, we examined the ability of (R)-Tiagabine and CI-966 to inhibit the uptake of [^{3}H]GABA; for comparison, we also examined these compounds at GAT-1. These compounds are lipophilic derivatives of

nipecotic acid and guvacine, respectively. As shown in Table 2, (R)-Tiagabine at a concentration of 100μM completely inhibits uptake at GAT-1 but has no effect at GAT-2 and GAT-3. Tiagabine is reported to have high potency at both neuronal and glial GABA transporters (6), and has demonstrated efficacy as an anticonvulsant in early clinical trials (8). The finding that Tiagabine has very low affinity for GAT-2 and GAT-3 underscores the potential of these transporters as unique drug targets. Similar to Tiagabine, the GABA uptake blocker CI-966 (72) displays far greater potency at GAT-1 than at GAT-2 and GAT-3 (Table 2). CI-966 was developed as an anticonvulsant but was withdrawn due to severe side effects observed in Phase 1 clinical trials (63).

Table 1. Ion Dependence of [^{3}H]GABA Uptake

	Uptake[a]		
Condition[a]	GAT-1	GAT-2	GAT-3
Na^+-free	0.5±0.3 (3)	0.1±0.06 (3)	0.3±0.03 (3)
Cl^--free	5±2 (3)	43.2±4.0 (5)	20.2±5.8 (5)

[a]COS-7 cells transfected with rB46a, rB14b, or rB8b were incubated for 10 minutes (37°C) with 50nM [^{3}H]GABA in either HBS, or in HBS in which Li^+ was substituted for Na^+ (Na^+-free), or in which acetate was substituted for Cl^- (Cl^--free). Non-specific uptake was determined with 1mM GABA. Data represent specific uptake, expressed as percent of uptake in HBS (mean ±SEM; values in parentheses indicate number of experiments).

Table 2. Pharmacological Specificity of [^{3}H]GABA Uptake

Inhibitor[a]	concen-tration	% Inhibition[a] GAT-1	GAT-2	GAT-3
ACHC[b]	100μM	49±10(3)	3±3(3)	0±0(3)
β-alanine	100μM	11±1(8)	86±1(8)	70±1(7)
betaine	500μM	0(2)	9(2)	1(2)
L-DABA	100μM	49±8(7)	43±8(7)	4±1(5)
guvacine	10μM	41±3(4)	13±1(3)	8±5(3)
OH-nipecotic	10μM	34±5(3)	9±7(3)	5±2(3)
nipecotic	10μM	51±5(3)	5±5(3)	12±6(3)
THPO	100μM	10(2)	9(2)	4(2)
(R)-Tiagabine	100μM	100±1(3)	0±1(3)	0±1(3)
CI-966	100μM	91±2(3)	9±6(3)	10±6(3)

[a]COS-7 cells transfected with rB46a, rB14b, or rB8b were incubated for 10 minutes (37°C) with 50nM [^{3}H]GABA and the indicated compounds. Non-specific uptake was determined with 1mM GABA. Data show percent displacement of specific [^{3}H]GABA uptake, mean ±SEM (values in parentheses indicate number of experiments).

[b] L-DABA = L-(2,4)diaminobutyric acid
THPO = 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol
ACHC = cis-3-aminocyclohexanecarboxylic acid
CI-966 = [1-[2-[bis 4-(trifluoromethyl)phenyl]methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid
Tiagabine= (R)-N-[4,4-bis(3-methyl-2-thienyl)but-3-en-1-yl]nipecotic acid

Tissue Localization Studies of Mammalian GABA Transporters:

To define the tissue distribution patterns of the novel GABA transporters, polymerase chain reaction (PCR) was used to detect each sequence in cDNA from seven different rat tissues. For comparison, the distribution of GAT-1 was also studied. Radiolabeled probes were used to detect individual PCR products by hybridization; each of the probes was highly specific for the transporter under study (data not shown). As shown in Figure 5B, GAT-1 was detectable in brain and retina but not liver, kidney, heart, spleen, or pancreas after 30 cycles of PCR. GAT-2 was present not only in brain and retina, but also in liver, kidney, and heart. Levels of GAT-2 mRNA were also detectable in spleen with overexposure of the autoradiogram (data not shown). Similar to GAT-1, the distribution of GAT-3 was limited to brain and retina. Cyclophilin was amplified to a similar extent from all the tissues (data not shown), indicating that adequate cDNA was present in each sample. Samples of poly A+ RNA not treated with reverse transcriptase and subjected to identical PCR conditions showed no hybridization with the transporter probes (not shown), indicating that the signals obtained with cDNA could not be accounted for by genomic DNA contamination. Thus, among the tissues examined, the distribution of GAT-3 is limited to the CNS, while GAT-2 has a wide peripheral distribution as well. These results are supported by Northern blot analyses of total RNA isolated from rat brain and liver; a single ≈2.4kb transcript hybridizing with GAT-2 is present in both liver and brain, while a ≈4.7kb transcript hybridizing with GAT-3 is detectable only in brain (Figure 5A).

Cellular Localization of GABA Transporter mRNAs:
Prior to the recent cloning of GABA transporters (4,21), pharmacological evidence suggested that multiple transporters contributed to the high-affinity GABA uptake observed in rat brain (30). Both neuronal and glial elements transport GABA, and preparations enriched in each cell type display differential sensitivities to inhibitors of GABA transport (5, 53, 61), suggesting the presence of distinct neuronal and glial GABA transporters. The ability to design neuronal- or glial- selective GABA uptake inhibitors would be a major advantage in the design of effective therapeutic agents. The GABA transporter cloned from rat brain, designated GAT-1 (21), displays a pharmacological profile consistent with a "neuronal"-type carrier. Our cloning of two additional GABA transporters from rat brain, GAT-2 and GAT-3 (previously termed Ggaba1 and Ggaba2, respectively), confirms the principle of heterogeneity in high-affinity GABA transporters. Further, the sensitivity of GAT-2 and GAT-3 to inhibition by ß-alanine distinguishes them from GAT-1, and raises the possibility that one or both represent "glial"-type transporters. The availability of three cloned high-affinity GABA transporters now provides the opportunity to begin to examine the relationship between the pharmacologically defined neuronal and glial subtypes, and the transporters encoded by the cloned genes.

The presence of mRNAs representing each of the three GABA transporters was investigated in primary cultures of embryonic rat brain neurons, astrocytes, and meningeal fibroblasts. Polymerase chain reaction (PCR) was used to amplify each sequence for detection with specific probes. As shown in Table 3, the messenger RNAs encoding each GABA transporter had a unique pattern of distribution.

GAT-1 mRNA was present in all three culture types, whereas GAT-3 mRNA was restricted to neuronal cultures. GAT-2 mRNA was present in both astrocyte and fibroblast cultures, but not in neuronal cultures. Thus, GAT-2 and GAT-3, which exhibit extremely similar pharmacological profiles, display non-overlapping cellular distribution patterns. GAT-1, which displays a "neuronal"-type pharmacology, is apparently not restricted to a neuronal distribution.

Table 3. Cellular Localization of GABA Transporters by PCR.

	Neuronal Cultures	Astrocyte Cultures	Fibroblast Cultures
GAT-1	+	+	+
GAT-2	-	+	+
GAT-3	+	-	-

Total RNA isolated from cultured embryonic rat neurons, astrocytes, or fibroblasts was converted to cDNA and subjected to PCR for detection of mRNAs encoding GAT-1, GAT-2, and GAT-3 as described in Experimental Procedures. Amplified products were separated on agarose gels, blotted to nylon membranes, and hybridized with radiolabeled oligonucleotides specific for each transporter cDNA. The blot was exposed to film and the autoradiogram developed after several hours. A (+) sign signifies that a positive signal was detected on the autoradiogram; a (-) signifies that no signal was detectable. The same results were observed in two independent experiments.

It is important to note that primary cultures, while enriched for a specific population of cells, may contain a small proportion of additional cell types. The sensitivity of PCR is sufficient to amplify a sequence contributed by a small number of cells; therefore, an unequivocal assignment of neuronal vs. glial localization would require combined *in situ* hybridization/immunocytochemistry. However, the presence of GAT-3 mRNA only in neuronal cultures suggests that detection of GAT-1 mRNA in astrocyte cultures is not due to the presence of contaminating neurons, and that GAT-1 is probably present in astrocytes in addition to neurons. The presence of GAT-1 and GAT-2 in fibroblast as well as astrocyte cultures may be explained by our recent finding that meningeal fibroblast cultures contain a large proportion of astrocytes as defined by staining with antibodies to glial fibrillary acidic protein (GFAP) (data not shown); thus, GAT-1 and GAT-2 signals in meningeal fibroblasts probably result from contaminating astrocytes.

These studies suggest that multiple high-affinity GABA transporter subtypes are present in different functional compartments, with at least two subtypes present in neurons (GAT-1 and GAT-3) and in glia (GAT-1 and GAT-2). Further, they indicate that pharmacologic agents selective for each subtype may have different therapeutic applications.

Localization of GAT-1 and GAT-3 mRNA by <u>in situ</u> Hybridization:

In situ hybridization of GAT-1 and GAT-3 was carried out using antisense probes to the 3' untranslated region and the 3,4 extracellular loop of each clone. Hybridization

of sense probes (control) to the same regions were also studied.

GAT-1 mRNA was observed in all rat brain areas examined (Table 4). In the telencephalon, the highest levels were observed in the glomerular layer of the olfactory bulb, the orbital cortex, the lateral septal nucleus, the ventral pallidum, the globus pallidus, amygdaloid area, and layer 4 of the cerebral cortex. Moderate levels were observed in the islands of Calleja, the internal and external plexiform layers, and the piriform, retrospenial, and cingulate cortices, as well as in all regions of the hippocampal formation.

In the diencephalon, the highest levels were found in the paraventricular and reticular thalamic nuclei, and in the dorsal lateral geniculate. Lower levels were seen in the reuniens and rhomboid thalamic nuclei. In the hypothalamus, moderate levels were seen in the suprachiasmatic and paraventricular nuclei, and in the medial preoptic area. Lower levels were seen in the supraoptic and anterior hypothalamic nuclei.

In the midbrain, high levels were seen in the substantia nigra (pars compacta and pars reticulata), median raphe, and the olivary pretectal nucleus. Lower levels were observed in the superior colliculus.

No label was seen in the pontine nuclei, nor in the cerebellar Purkinje cells.

GAT-3 mRNA was observed throughout the neuraxis (Table 5). Within the telencephalon, the highest levels were detected in the medial septal nucleus, the nucleus of the diagonal band, and the ventral pallidum. Lower levels

were found in the amygdala and the shell of the nucleus accumbens. Low levels were observed in the hippocampus. No labeling above background was observed in the neocortex.

In the thalamus, many nuclear groups were labeled. The areas with the highest labeling were the xiphoid, paraventricular, and rhomboid nuclei, and the zona incerta. Lower levels were observed in the following nuclei: reuniens, reticular, medial and lateral ventral posterior, and the medial geniculate. In the hypothalamus, moderate labeling was found in the lateral and ventromedial regions. Lower levels were observed in the arcuate nucleus and median eminence.

In the midbrain, the highest levels were observed in the dorsal tegmentum.

In the metencephalon, the highest levels were found in the medial vestibular and deep cerebellar nuclei, and lower levels in the lateral superior olivary nucleus. No label was observed in the cerebellar cortex.

A comparison of the localization of GAT-1 and GAT-3 mRNAs indicates that both are widely distributed in the brain, and while GAT-1 is more abundant on a per cell basis, the two tend to have overlapping distributions. Notable exceptions are cortex and hippocampus which contain large numbers of neurons containing GAT-1 mRNA but few cells with GAT-3 mRNA. On the other hand, GAT-3 mRNA levels appear to be higher than GAT-1 in the superficial layers of the superior colliculus and in the deep cerebellar nuclei.

Table 4. In situ localization of GAT-1 in the Rat CNS

Area[1]	Labeling[2]	
	Probe 191 AS 3'UT	Probe 179 AS 3,4 loop
BREGMA 6.20mm		
mitral cells	-	-
glomerular layer	++	++
ext.plexiform layer	+½	+
ant. olf nerve	+/-	+/-
BREGMA 5.20mm		
ext.plexiform layer	+	+
int.plexiform layer	+	+
ant.comm.intrabulb	+/-	+/-
AOM,D,V	+	+
orbital cortex m,v,l	+½	+½
frontal. cortex	+	+½
BREGMA 1.60mm		
tenia tecta	+	+
lat.septal nucleus	+/-	+/-
lat.septal interm.	++	½+
ICjM	+½	+½
caudate-putamen	+/-	-
AcbSh	+	½+
AcbC	½+	-
vent.pallidum	+++	+++
olf.tubercle	-	-
ICj	+	+
piriform ctx.	+	+
cingulate ctx	+	+
indusium griseum	++	+½
BREGMA-1.40mm		
retrosplen.ctx	+	½+
cortex I	+	+
IV	++	++
V	+	+
reticular thal.nu.	+½	+½
globus pallidus	+++	++½
caudate-putamen	+	+
ant.dor thal.nu.	-	-
paraventr. thal. nu	+½	+½
supraoptic nu.	½+	½+
suprachiasmatic nu.	+	+
med.preoptic area	+½	+½

Table 4 (continued)

Area[1]	Labeling[2]	
	Probe 191 AS 3'UT	Probe 179 AS 3,4 loop
perivent. hypoth. nu.	+	+
anter. hypoth. nu.	+	+
paravent. hypoth. nu.	+½	+½
nu. horizontal. limb diag. band	+	+
ant. amygd. area	++½	++½
BREGMA -1.80mm		
reuniens thal.nu.	½+	½+
rhomboid thal.nu.	½+	½+
retrochiasmatic area	+	+
BREGMA -4.52mm		
choroid plexus	-	-
PMCo	+	+
AHiA	+	+
Basolateral Amygdaloid nu.	++	++
dorsal endopiriform nu.	+	+
hippocampus (all levels)	+.	+
polymorphic dendate gyrus	++	++
olivary pretectal nu.	++	++
dorsal lateral genicul. nu.	++	++
BREGMA -5.30mm		
substantia nigra		
pars reticulata	++	++½
pars compacta	++	++
red nucleus parvocellular	-	-
retrospenial cortex	+	+
occipital cortex	+	+
nucleus Darkschewitsch	+½	+
nucleus posterior commis., magnocellular	+	+½
BREGMA -7.64mm		
superior colliculus	+	+
central grey	-	-
dorsal grey	+/-	+/-
median Raphe	+½	+½
pontine nuclei	-	-
Purkinje cells	+/-	+/-

[1] abbreviations as in Paxinos, G. and Watson, C. (1986) The Rat Brain in Stereotactic Coordinates, second edition. Academic Press.

Table 4 (continued)

2 Antisense probes 191 and 179 were to 3' untranslated region and to the 3,4 extracellular loop, respectively. Control data using sense probes to the same regions showed no labeling.

Labeling scale: -, no labeling; ½+, very weak, +, weak; ++, moderate; +++, heavy. Note that the scale is based on maximal labeling obtained with GAT-1 probes and should not be compared to results for GAT-3.

Table 5. In situ Localization of GAT-3 in the Rat CNS

Area[1]	Labeling
telencephalon:	
cortex	-
piriform ctx	½+
nu. accumbens	
core	-
shell	+
olf. tubercle	½+
med. septal nu.	++
nu. horiz.limb	
diag. band	++
ventral pallidum	++
ant. cortical amygdaloid nu.	+
medial amygdaloid nu.	+½
Diencephalon:	
paraventricular thalamic nu.	++½
reticular thalamic nu.	+½
VPM	+½
VPL	+
zona incerta	++½
rhomboid thalamic nu.	++½
reuniens thalamic nu.	++
xiphoid thalamic nu.	+++
medial geniculate nu.	+
arcuate hypoth. nu.	½+
ventromedial hypoth.nu.	+
lateral hypoth. nu.	+½
median eminence	½+
hippocampus	½+
Mesencephalon:	
superior colliculus	++½
central gray, dorsal	++
central gray	++
substantia nigra	not examined
interpeduncular nu.	
caudal	+
dorsal raphe	+
cuneiform nu.	+
lateral dorsal tegmen. nu.	+++
dorsal tegmental nu.,	
pericentral	+++

Table 5. (continued)

Area[1]	Labeling[2]
Metencephalon:	
medial vestibular nu.	+++
lateral superior olive	++
inferior olive	not examined
cerebral cortex	-
deep cerebellar nuclei	+++

[1] abbreviations as in Paxinos, G. and Watson, C. (1986) The Rat Brain in Stereotactic Coordinates, second edition. Academic Press.

[2] Data are pooled from antisense probes to the 3' untranslated region and to the 3,4 extracellular loop. Control data using sense probes to the same regions showed no labeling.

Labeling scale: -, no labeling; ½+, very weak, +, weak; ++, moderate; +++, heavy. Note that the scale is based on maximal labeling obtained with GAT-3 probes and should not be compared to results for GAT-1.

Discussion

The recent cloning of transporters for GABA (21), norepinephrine (55), dopamine (33,65), serotonin (3,23), glycine (68), and taurine (66) has helped to define the structural properties of this class of membrane proteins. In contrast with neurotransmitter receptors, however, it has not been determined for neurotransmitter transporters whether multiple subtypes exist and/or play a role in synaptic transmission. Our identification of two cDNA clones from rat brain encoding novel GABA transporters (designated GAT-2 and GAT-3) provides the first molecular evidence for heterogeneity within the neurotransmitter transporter gene family, and raises the possibility that multiple GABA transporters participate in the regulation of GABAergic neurotransmission.

Both proteins have 12 putative tránsmembrane domains and can be modeled with a similar topology to the neuronal GABA transporter (GAT-1; (21)), including a large glycosylated extracellular loop between TMs 3 and 4. Analysis of amino acid homologies of the various transporters reveals some unexpected relationships. For example, GAT-2 and GAT-3 exhibit greater amino acid sequence identity to each other (67%) than to GAT-1 (~53%), despite all three transporters displaying nearly identical affinities for GABA. Surprisingly, the sequence closest to GAT-2 and GAT-3 is the dog betaine transporter (79) which, in fact, is as homologous to GAT-2 and GAT-3 as they are to one another. Significantly, the cloned betaine transporter has also been reported to transport GABA (79), although the affinity of GABA at the betaine transporter is nearly 10-fold lower than at GAT-2 and GAT-3. Conversely, the betaine transporter displays at least 10-fold higher affinity for betaine than do GAT-

2 and GAT-3 (see Table 2). Thus, transporters with as little as 53% amino acid homology can display high affinity for the same substrate (eg. GAT-1 vs. GAT-2 and GAT-3), whereas transporters only slightly more divergent can demonstrate markedly different substrate specificities (eg., GAT-1 vs. glycine, 45% homology; (68)).

Pharmacologically distinct GABA transporters have previously been identified in neuronal and glial cell cultures (15, 36 and 62). Thus, it was of interest to examine the sensitivity of GAT-2 and GAT-3 to a variety of inhibitors and to compare this to published values for endogenous transporters in primary cell cultures, as well as to GAT-1. It is noteworthy that GAT-2 and GAT-3 display greater sensitivity to the glial-selective drug β-alanine than does the previously cloned GAT-1, suggesting similarity to the tranporter(s) characterized in glial cell cultures. However, a lack of identity with the pharmacologically defined glial-type transporter is demonstrated by the finding that guvacine, nipecotic acid, Tiagabine, and hydroxynipecotic acid are much less potent inhibitors of GABA uptake at GAT-2 and GAT-3 than at the transporter present in glial cultures (6, 15, 36, 62). Additionally, these compounds are more potent in neuronal cultures (and at the previously cloned GAT-1) than at GAT-2 and GAT-3, which also distinguishes the newly cloned transporters from the neuronal GABA transporter (6, 15, 21, 36 and 62). Lastly, although GAT-2 and GAT-3 display similar sensitivity to a number of the inhibitors examined and show similar affinity for GABA itself, they can be distinguished by L-DABA, which displays higher potency at GAT-2 than at GAT-3. Interestingly, the potency of L-DABA at GAT-2 is similar to that of GAT-1 (Table 2), blurring the distinction

between the newly cloned tranporters and the neuronal-type transporter. This finding may indicate that a spectrum of GABA transport activities underlie the neuronal and glial profiles observed in tissue preparations. Lastly, the three cloned GABA transporters can also be distinguished by their differential dependence on external chloride: GAT-1 is the most chloride dependent, GAT-2 the least, and GAT-3 is intermediate in its sensitivity. The finding that GABA transport by GAT-2 and GAT-3 is not completely eliminated in chloride-free medium suggests that their mechanism of transport is fundamentally different from that of GAT-1.

It is somewhat surprising that the pharmacological profiles of GAT-2 and GAT-3 differ from those of previously characterized transporters in neuronal and glial cultures. One possible explanation is that the unique pharmacology of GAT-2 and GAT-3 reflects species differences, as the cloned transporters were obtained from a rat cDNA library, while mouse tissue was employed in many of the earlier studies (15, 36 and 62). This hypothesis gains validity from the finding that certain GABA uptake blockers are potent anticonvulsants in rats, but are ineffective in mice (82), although differences in drug metabolism or distribution have not been ruled out. A second possibility is that since neuronal and glial cultures are prepared from fetal or newborn animals, the discrepant results may reflect developmental changes in GABA transporters or peculiarities of glia and neurons when maintained in cell culture. Alternatively, the two newly cloned transporters may in fact represent members of a novel class of transporters that have not been previously identified, perhaps due to their low abundance in cultured cells. This would suggest that further GABA transporters with pharmacological profiles consistent

with those seen in neuronal and glial cultures remain to be cloned. Lastly, it should be pointed out that the pharmacological profiles of cloned transporters for serotonin (3,23), dopamine (33,65), and norepinephrine (55), as well as GAT-1 are similar to those observed in brain homogenates, thus arguing that the unique properties of GAT-2 and GAT-3 are not the result of the heterologous expression system.

Despite the generally similar pharmacology of GAT-2 and GAT-3, their patterns of distribution are distinct. All three high-affinity GABA transporters are present in brain and retina, while only GAT-2 was detected in peripheral tissues. This finding is consistent with recent studies suggesting a role for GABA in liver (52), kidney (1,19) and other peripheral tissues (for review, ref. 14). Further distribution studies of GAT-2 and GAT-3 by *in situ* localization of transporter mRNAs in conjunction with immunocytochemistry will help to define the roles of these transporters in GABAergic transmission.

In conclusion, we now report the identification in mammalian brain of two novel high-affinity GABA transporters with unique pharmacological properties. These studies indicate previously unsuspected complexity in the regulation of GABAergic transmission, and provide the opportunity for the development of selective therapeutic agents to treat neurological and psychiatric disorders.

Cloning of Human High-Affinity GABA Transporters:
The use of human gene products in the process of drug development offers significant advantages over those of other species, which may not exhibit the same

pharmacologic profiles. To facilitate this human-target based approach to drug design in the area of inhibitory amino acid transporters, we used the nucleotide sequences of the rat GAT-2 and GAT-3 cDNAs to clone the human homologues of each gene.

To obtain a cDNA clone encoding the human GAT-2 GABA transporter (hGAT-2) we used PCR primers based on the rat GAT-2 sequence to detect the presence of hGAT-2 in human cDNA libraries. PCR was carried out at a reduced annealing temperature to allow mismatches between rat and human sequences (see Experimental Procedures); amplified hGAT-2 sequences were detected by hybridization at low stringency with radiolabeled (randomly primed) rat GAT-2 cDNA. A human heart cDNA library (Stratagene) was identified and screened at low stringency with the same probe, resulting in isolation of a partial cDNA clone (hHE7a) containing the C-terminal portion of the coding region of hGAT-2. Using human sequence derived from this clone, a partial cDNA clone (hS3a) was isolated from a human striatum cDNA library (Stratagene) that provided additional sequence in the coding region. The hGAT-2 nucleotide sequence from these two clones and the deduced amino acid sequence based on translation of a long open reading frame is shown in Figure 10A. The sequence includes 738 base pairs of coding region (246 amino acids) and 313 base pairs of 3' untranslated region. Comparison with the rat GAT-2 amino acid sequence reveals 90% identity over the region encoded by the clones, which includes predicted transmembrane domains 8-12 and the carboxy terminus of hGAT-2.

To obtain the nucleotide sequence of the human GAT-3 GABA transporter (hGAT-3), degenerate PCR primers were used to amplify transporter sequences from human cDNA libraries.

Amplified hGAT-3 sequences were detected in the library by hybridization at low stringency with radiolabeled oligonucleotides representing the region of the rat GAT-3 cDNA that encodes a portion of the second extracellular loop. The human fetal brain library (Stratagene) identified by this approach was screened at high-stringency with the same probes; positive plaques were purified by successive screening at low stringency. Two cDNA clones were isolated (hFB16a, hFB20a) which together comprise nearly the entire coding region of hGAT-3; the sequence of the remaining 7 base pairs was supplied by a genomic clone (hp28a) isolated from a human placental library. A vector comprising the complete coding sequence of hGAT-3 was constructed using appropriate fragments of these three clones, and is designated pcEXV-hGAT-3. The complete nucleotide sequence and predicted amino acid sequence of hGAT-3 are shown in Figure 10B. In addition to 1896 base pairs of coding region, the sequence includes 5' and 3' untranslated sequence (34 and 61 base pairs, respectively). Translation of a long open reading frame predicts a protein of 632 amino acids that is 95% identical to the rat GAT-3 and contains 12 putative transmembrane domains. Methods similar to methods used to clone the human homologues of the mammalian GABA transporters can similarly be used to clone the human homologues of the mammalian taurine transporter.

The cloning and expression of the human GAT-2 and GAT-3 will allow comparison of pharmacological profiles with those of rat GABA transporters, and also provide a means for understanding and predicting the mechanism of action of GABA uptake inhibitors as human therapeutics. Recently, several additional transporters have been cloned which exhibit significant sequence homology with

previously cloned neurotransmitter transporters. cDNA and genomic clones representing the mouse homologues of GAT-1 were recently reported (39). In addition, a glycine transporter cDNA that is similar but not identical to that cloned by Smith et al. (68) was cloned from both rat (22) and mouse (39). A high-affinity L-proline transporter was reported by Fremeau et al.(18), supporting a role for L-proline in excitatory neurotransmission. A rat cDNA identified as a choline transporter was reported by Mayser et al. (50). A taurine transporter cDNA was recently cloned from dog kidney cells (74) which is 90% identical to the rat taurine transporter amino acid sequence reported by Smith et al. (66). A cDNA encoding a mouse GABA transporter was recently cloned by Lopez-Corcuera et al. (45); the transporter encoded by this cDNA is 88% identical to the dog betaine transporter (79), and may represent the mouse homologue of that gene. Finally, a β-alanine-sensitive GABA transporter from rat brain has been cloned (10) that exhibits 100% amino acid identity with the rat GAT-3 sequence reported by Borden et al. (4).

2. Taurine

Results and Discussion

Cloning of Mammalian Taurine Transporter:

We screened a rat brain cDNA library at low stringency with probes encoding the rat brain GABA transporter GAT-1 (21) in order to identify additional inhibitory amino acid transporter genes. Several clones were isolated which hybridized at low but not at high stringency with the GABA transporter probes. Characterization of the clones by DNA sequence analysis revealed that they represented a novel transporter sequence related to GAT-1. None of the clones contained the complete coding region of the putative transporter, and thus the library

was rescreened at high stringency using oligonucleotides designed from the new sequence. A 2.5 kb cDNA clone (designated rB16a) was isolated which contained an open reading frame of 1863 base pairs encoding a protein of 621 amino acids (Figure 1C). Comparison of this sequence with the rat GABA transporter cDNA revealed 58% nucleotide identity within the coding region. Comparison with sequences in Genbank and EMBL data bases demonstrated that the sequence was novel and that the most closely related sequence was the rat GABA transporter (21) followed by the human norepinephrine transporter (55). Subsequent comparisons to recently cloned transporters indicate that the most homologous sequences are two novel GABA transporters designated GAT-2 and GAT-3 (4) and the betaine transporter (79), which exhibit 62-64% nucleotide identity with rB16a.

The amino acid sequence deduced from the nucleotide sequence of rB16a is shown in Figure 1E with a membrane topology similar to that proposed for the rat GABA transporter (21) and other cloned neurotransmitter transporters (3, 23, 33, 55 and 65). The translation product of rB16a is predicted to have a relative molecular mass of ~70,000 Daltons. Hydropathy analysis indicates the presence of 12 hydrophobic domains which may represent membrane spanning segments. Three potential sites for Asn-linked glycosylation are found in the extracellular loop between the third and fourth transmembrane domains. Alignment of the deduced amino acid sequence of rB16a with the rat GABA transporter (GAT-1; (21)) and the dog betaine transporter (79) revealed 50% and 58% amino acid identities, respectively (Figure 6). Comparison of rB16a with the glycine transporter (Figure 6; (68)) and the human norepinephrine transporter (55) also showed significant amino acid

homology (41-45%), similar to that between GAT-1 and the norepinephrine transporter (46%). As predicted from nucleotide comparisons, the strongest amino acid homology (~61%) is with the GABA transporters GAT-2 and GAT-3 recently cloned from rat brain (4). In contrast, the sodium/glucose cotransporter (22), which shows a low degree of homology with cloned neurotransmitter transporters, displays only 21% amino acid identity with rB16a. These data suggested that the new sequence might encode an inhibitory amino acid transporter expressed in the brain. To explore this possibility, rB16a was placed in a mammalian expression vector, transfected into COS cells, and screened for transport of a variety of radiolabeled neurotransmitters and amino acids.

Pharmacological Characterization of Mammalian Taurine Transporter:

COS cells transiently transfected with rB16a (COS/rB16a) accumulated approximately 6-fold more [^{3}H]taurine than control, non-transfected cells (Figure 7). Specific uptake represented greater than 95% of total uptake in transfected cells. In contrast, the uptake of [^{3}H]glutamate, [^{3}H]glycine, [^{3}H]5-HT, [^{3}H]dopamine, and [^{3}H]GABA was unaltered. Uptake of [^{3}H]taurine was not observed following mock transfection, indicating that the enhanced uptake was not the result of non-specific perturbation of the membrane. The transport of [^{3}H]taurine by COS/rB16a was decreased >95% when Na^+ was replaced by Li^+, or when Cl^- was replace by acetate (Figure 7). In the absence of sodium or chloride, taurine transport in COS/rB21a decreased to levels below that of non-transfected controls, demonstrating that endogenous taurine transporter activity present in COS cells is also dependent on these ions. A similar ion dependence has been observed for taurine transport *in*

vivo (27), as well as for the activity of other cloned neurotransmitter transporters such as those for GABA (21), glycine (68), and norepinephrine (55).

To determine the affinity of taurine for the cloned transporter, COS/rB16a was incubated with various concentrations of [^{3}H]taurine and the specific accumulation of radioactivity was determined. Accumulation of [^{3}H]taurine was dose-dependent and reached saturation at higher concentrations (Figure 8). Non-linear regression analysis of the data yielded the following values: K_M = 43±6 μM, and V_{MAX}= 0.96±0.27 nmoles/mg protein (mean ± SEM, n=4 experiments). The affinity of the cloned transporter for taurine is similar to that of high-affinity taurine transporters in both the central nervous system (42,80) and peripheral tissues (37) which exhibit K_M values from 10 to 60 μM. Taken together, these data indicate that rB16a encodes a saturable, high-affinity, sodium- and chloride-dependent taurine transporter.

To determine the pharmacological specificity of the cloned transporter, various agents were examined for their ability to inhibit the transport of [^{3}H]taurine by COS/rB16a (Table 6). As the endogenous taurine transporter in COS cells accounted for, on average, 16% of the total transport activity observed in transfected cells, we were concerned that this could influence results. Accordingly, we also examined the sensitivity of the endogenous taurine transporter present in non-transfected cells. As shown in Table 6, the pharmacologic properties of the cloned taurine transporter closely matched those of the endogenous transporter and thus did not lead to erroneous results.

The most potent inhibitors were taurine and hypotaurine, each of which inhibited specific [^{3}H]taurine uptake approximately 30-40% at 10μM, 90% at 100μM, and 100% at 1mM. β-alanine was slightly less potent, inhibiting specific uptake 15%, 51%, and 96% at 10μM, 100μM, and 1mM, respectively; the high potency of β-alanine as an inhibitor of taurine uptake is consistent with the finding that COS/rB16a showed a 6-fold increase in the specific uptake of [^{3}H]β-alanine (data not shown), essentially identical to the fold-increase observed with [^{3}H]taurine. The taurine analogue GES was also quite potent, inhibiting specific uptake of [^{3}H]taurine 11%, 45% and 92% at 10μM, 100μM and 1mM, respectively. APSA and GABA both inhibited uptake approximately 10% and 40% at 100μM and 1mM, respectively. The observations that GABA is a poor inhibitor of taurine uptake, and that transfection with rB16a did not result in enhanced uptake of [^{3}H]GABA (see above), are consistent with the previous report (38) that GABA is a weak non-competitive inhibitor of taurine uptake. Less than 10% inhibition of [^{3}H]taurine uptake was observed for the following compounds (each tested at 1mM): the structural analogues AEPA and MEA as well as the sulfur-containing amino acids cysteine and methionine (Table 6), and (data not shown) norepinephrine, dopamine, glutamate, glycine, serine, betaine, L-methionine, and α-methylaminoisobutyric acid (a substrate for amino acid transporter designated system A; (21)). Taken together, these results indicate that the taurine transporter encoded by rB16a is similar to the endogenous taurine transporter in COS cells (Table 6), as well as the endogenous taurine transporter(s) present in neural tissue (25),(see also ref. 27 and references therein).

It is of interest that sensitivity to β-alanine is shared by the two high-affinity GABA transporters recently cloned from rat brain (GAT-2 and GAT-3 (4)), which are even more closely related to the taurine transporter (62% amino acid identity) than to the neuronal-type GABA transporter GAT-1 (51%). β-alanine has been shown to activate an inward chloride current in spinal neurons (9,49) and is released in a calcium-dependent manner from several brain areas (31,58), suggesting a role as an inhibitory neurotransmitter in the CNS. The similar sensitivities of the newly cloned GABA transporters [4] and the taurine transporter to β-alanine, combined with their sequence homologies, suggest that they represent a subfamily of inhibitory neurotransmitter transporters. Despite these similarities, these transporters unexpectedly exhibit widely divergent affinities for GABA: GAT-2 and GAT-3 show the highest affinity (Km=10μM (4)), while the affinity of the taurine transporter is extremely low (~1mM, Table 6). Interestingly, the dog betaine transporter (79), which displays a similar degree of homology to the members of this subfamily (ca. 60%), exhibits an intermediate affinity for GABA (~100μM). The finding that four structurally related transporters display overlapping substrate specificities for the neuroactive amino acids GABA and β-alanine suggests that multiple transporters may regulate the synaptic levels of these substances. This crossreactivity underscores the importance of understanding the action of therapeutic agents at both GABA and taurine transporters.

Table 6. Pharmacological Specificity of [^{3}H]taurine Uptake.

Inhibitor[a]	Concentration	% Inhibition	
		control	rB16a
AEPA	1mM	0±0 (4)	3±3 (5)
AMSA	1mM	1±1 (4)	7±3 (4)
APSA	100μM	7±3 (4)	8±4 (4)
	1mM	45±3 (5)	36±4(5)
β-alanine	10μM	9±2 (6)	15±6(6)
	100μM	63±3 (6)	51±4(10)
	1mM	97±1 (4)	96±1 (8)
CSA	1mM	2±1 (4)	7±5 (3)
cysteine	1mM	4±3 (3)	2±2 (3)
GABA	10μM	1±1 (4)	9±6 (4)
	100μM	9±4 (6)	10±4 (10)
	1mM	49±2 (5)	44±6(8)
GES	10μM	6±3 (4)	11±4 (4)
	100μM	47±3 (5)	45±5 (5)
	1mM	89±1 (5)	92±1 (6)
hypotaurine	10μM	41±3 (7)	26±7 (7)
	100μM	91±1 (4)	84±3 (4)
	1mM	99±1 (4)	100±1 (4)
MEA	1mM	1±0 (3)	3±3 (4)
methionine	1mM	1±1 (3)	1±1 (3)

Table 6 (continued)

taurine	10μM	38±5 (7)	29±8 (5)
	100μM	89±2 (4)	83±2 (5)
	1mM	100[b]	100[b]

a Non-transfected COS-7 cells (control), or COS-7 cells transfected with rB16a were incubated for 10 minutes (37°C) with 50nM [^{3}H]taurine and the indicated compounds. Data show percent displacement of specific [^{3}H]taurine uptake (mean±SEM; values in parentheses indicate number of experiments).

b Non-specific uptake defined with 1mM taurine.

Abbreviations: AEPA, 2-aminoethylphosphonic acid; AMSA, aminomethanesulfonic acid; APSA, 3-amino-1-propanesulfonic acid; CSA, cysteinesulfinic acid; GABA, gamma-aminobutyric acid; GES, guanidinoethanesulfonic acid; MEA, 2-mercaptoethylamine.

Tissue Localization Studies of Mammalian Taurine Transporter:

To define the tissue distribution patterns of the taurine transporter, polymerase chain reaction (PCR) was used to detect the rB16a sequence in cDNA representing mRNA from seven different rat tissues. As a control, the distribution of the constitutively expressed protein cyclophilin was also examined. Radiolabeled oligonucleotides specific for rB16a were used to detect PCR products by hybridization. As shown in Figure 9A, the taurine transporter was detectable in all tissues examined, including brain, retina, liver, kidney, heart, spleen, and pancreas, after 30 cycles of PCR. Cyclophilin was amplified to a similar extent from all the tissues (data not shown), demonstrating that adequate cDNA was present in each sample.

To evaluate both the abundance and the size of the mRNA encoding the taurine transporter, Northern blot analysis was carried out on poly A+ RNA isolated from the same rat tissues used for PCR analysis, with the addition of lung. As shown in Figure 9B, a single ~6.2 kb transcript which hybridized with the taurine transporter cDNA probe was detected in brain, kidney, heart, spleen, and lung after an overnight exposure of the autoradiogram. After a 3-day exposure, bands of the same size were also visible in liver and pancreas (data not shown). Rehybridization of the blot with the cDNA encoding cyclophilin (12) confirmed that roughly equal amounts of RNA were present in each sample except that of retina, which was significantly degraded (data not shown). Thus, taurine transporter mRNA levels were highest in brain and lung, intermediate in kidney, heart, and spleen, and lowest in liver and pancreas. The abundance and pattern of distribution of the taurine transporter mRNA by Northern

blot are consistent with data obtained using PCR (Figure 9); further, the same size transcript is present in all tissues evaluated. These findings suggest that a single taurine transporter functions in both the brain and peripheral tissues; however, we can not exclude the existence of additional taurine transporters.

Taurine is abundant in the central nervous system and is involved in a variety of neural activities. Unlike classical neurotransmitters, the effects of taurine are mediated both intra- and extracellularly. A major regulator of taurine levels, both within cells and in the synaptic cleft, is the transport of taurine across the plasma membrane. Our cloning of a high-affinity taurine transporter represents a critical step in defining the role of taurine in both neural and non-neural tissues, and in the development of therapeutic agents that alter taurine and GABA neurotransmission. In addition, the identification of a new member of the set of inhibitory amino acid transporters will aid in elucidating the molecular structure-function relationships within the transporter family.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Smith, E. Kelli
Borden, A. Laurence
Hartig, R. Paul
Weinshank, L. Richard

(ii) TITLE OF INVENTION: DNA ENCODING TAURINE AND GABA TRANSPORTERS AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.24

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: White, John
(B) REGISTRATION NUMBER: 28,678
(C) REFERENCE/DOCKET NUMBER: 40558A

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: 212-664-0525
(C) TELEX: 422523 COOP UI

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2028 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(v) FRAGMENT TYPE: N-terminal
(vii) IMMEDIATE SOURCE:
(A) LIBRARY: rat brain

(B) CLONE: rB14b

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 126..1932
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Lys Pro Val Cys Pro Val Met Glu Lys Val Glu Glu Asp Gly Thr Leu
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GAA CGG GAG CAA TGG ACC AAC AAG ATG GAG TTC GTA CTG TCA GTG GCG       263
Glu Arg Glu Gln Trp Thr Asn Lys Met Glu Phe Val Leu Ser Val Ala
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Gly Glu Ile Ile Gly Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys
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Tyr Lys Asn Gly Gly Gly Ala Phe Phe Ile Pro Tyr Leu Ile Phe Leu
         65                  70                  75

TTT ACC TGT GGC ATT CCT GTC TTC TTC CTG GAG ACA GCG CTT GGC CAG       407
Phe Thr Cys Gly Ile Pro Val Phe Phe Leu Glu Thr Ala Leu Gly Gln
     80                  85                  90

TAC ACC AAC CAG GGA GGC ATC ACA GCC TGG AGG AAA ATC TGT CCC ATC       455
Tyr Thr Asn Gln Gly Gly Ile Thr Ala Trp Arg Lys Ile Cys Pro Ile
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TTC GAG GGC ATC GGC TAT GCC TCA CAG ATG ATC GTC AGC CTT CTC AAT       503
Phe Glu Gly Ile Gly Tyr Ala Ser Gln Met Ile Val Ser Leu Leu Asn
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GTC TAC TAC ATC GTT GTC CTG GCC TGG GCC CTC TTC TAC CTC TTC AGC       551
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AGC TTC ACC ACT GAC CTC CCC TGG GGT AGC TGC AGC CAC GAG TGG AAT       599
Ser Phe Thr Thr Asp Leu Pro Trp Gly Ser Cys Ser His Glu Trp Asn
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GAG CTG GTC CTG TGC CTC CTG CTT GCC TGG ATC ATC TGC TAT TTC TGC      791
Glu Leu Val Leu Cys Leu Leu Leu Ala Trp Ile Ile Cys Tyr Phe Cys
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ACA CGT CTG TGG GAT CCC CAG GTG TGG ATG GAT GCG GGC ACC CAG ATC      983
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Phe Phe Ser Phe Ala Ile Cys Leu Gly Cys Leu Thr Ala Leu Gly Ser
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Tyr Asn Lys Tyr His Asn Asn Cys Tyr Arg Asp Cys Val Ala Leu Cys
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ATT CTC AAC AGC AGC ACC AGC TTC GTG GCC GGG TTT GCC ATC TTC TCC     1127
Ile Leu Asn Ser Ser Thr Ser Phe Val Ala Gly Phe Ala Ile Phe Ser
    320                 325                 330

ATC CTG GGC TTC ATG TCT CAG GAG CAG GGC GTA CCC ATA TCT GAG GTT     1175
Ile Leu Gly Phe Met Ser Gln Glu Gln Gly Val Pro Ile Ser Glu Val
335                 340                 345                 350

GCT GAA TCA GGC CCT GGC CTG GCA TTC ATC GCC TAC CCT CGA GCT GTG     1223
Ala Glu Ser Gly Pro Gly Leu Ala Phe Ile Ala Tyr Pro Arg Ala Val
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Val Met Leu Pro Phe Ser Pro Leu Trp Ala Cys Cys Phe Phe Phe Met
            370                 375                 380

GTG GTT CTC CTG GGA CTA GAC AGC CAG TTT GTG TGT GTA GAA AGC CTC     1319
Val Val Leu Leu Gly Leu Asp Ser Gln Phe Val Cys Val Glu Ser Leu
        385                 390                 395

GTG ACA GCG CTG GTG GAC ATG TAT CCC CGG GTG TTC CGT AAG AAG AAC     1367
Val Thr Ala Leu Val Asp Met Tyr Pro Arg Val Phe Arg Lys Lys Asn
    400                 405                 410

CGG AGG GAG ATT CTC ATC CTC ATC GTG TCT GTC GTC TCT TTC TTC ATC     1415
Arg Arg Glu Ile Leu Ile Leu Ile Val Ser Val Val Ser Phe Phe Ile
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Asp Tyr Tyr Ala Ala Ser Gly Met Cys Leu Leu Phe Val Ala Ile Phe
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GAG TCC CTC TGT GTG GCT TGG GTT TAC GGA GCC AGC CGC TTC TAT GAC      1559
Glu Ser Leu Cys Val Ala Trp Val Tyr Gly Ala Ser Arg Phe Tyr Asp
        465                 470                 475

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Asn Ile Glu Asp Met Ile Gly Tyr Lys Pro Trp Pro Leu Ile Lys Tyr
    480                 485                 490

TGT TGG CTC TTT TTC ACG CCA GCT GTG TGC CTG GCA ACC TTC CTG TTC      1655
Cys Trp Leu Phe Phe Thr Pro Ala Val Cys Leu Ala Thr Phe Leu Phe
495                 500                 505                 510

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Ser Leu Ile Lys Tyr Thr Pro Leu Thr Tyr Asn Lys Lys Tyr Thr Tyr
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Pro Trp Trp Gly Asp Ala Leu Gly Trp Leu Leu Ala Leu Ser Ser Met
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Val Cys Ile Pro Ala Trp Ser Ile Tyr Lys Leu Arg Thr Leu Lys Gly
        545                 550                 555

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Pro Leu Arg Glu Arg Leu Arg Gln Leu Val Cys Pro Ala Glu Asp Leu
    560                 565                 570

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Pro Gln Lys Ser Gln Pro Glu Leu Thr Ser Pro Ala Thr Pro Met Thr
575                 580                 585                 590

TCC CTC CTC AGG CTC ACA GAA CTG GAG TCT AAC TGC T AGGGACGAGG         1942
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 602 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Asn Arg Val Ser Gly Thr Thr Ser Asn Gly Glu Thr Lys Pro
1               5                   10                  15

Val Cys Pro Val Met Glu Lys Val Glu Glu Asp Gly Thr Leu Glu Arg
            20                  25                  30

Glu Gln Trp Thr Asn Lys Met Glu Phe Val Leu Ser Val Ala Gly Glu
        35                  40                  45

Ile Ile Gly Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Lys
    50                  55                  60

Asn Gly Gly Gly Ala Phe Phe Ile Pro Tyr Leu Ile Phe Leu Phe Thr
65                  70                  75                  80

Cys Gly Ile Pro Val Phe Phe Leu Glu Thr Ala Leu Gly Gln Tyr Thr
                85                  90                  95

Asn Gln Gly Gly Ile Thr Ala Trp Arg Lys Ile Cys Pro Ile Phe Glu
            100                 105                 110

Gly Ile Gly Tyr Ala Ser Gln Met Ile Val Ser Leu Leu Asn Val Tyr
        115                 120                 125

Tyr Ile Val Val Leu Ala Trp Ala Leu Phe Tyr Leu Phe Ser Ser Phe
    130                 135                 140

Thr Thr Asp Leu Pro Trp Gly Ser Cys Ser His Glu Trp Asn Thr Glu
145                 150                 155                 160

Asn Cys Val Glu Phe Gln Lys Thr Asn Asn Ser Leu Asn Val Thr Ser
                165                 170                 175

Glu Asn Ala Thr Ser Pro Val Ile Glu Phe Trp Glu Arg Arg Val Leu
            180                 185                 190

Lys Ile Ser Asp Gly Ile Gln His Leu Gly Ser Leu Arg Trp Glu Leu
        195                 200                 205

Val Leu Cys Leu Leu Leu Ala Trp Ile Ile Cys Tyr Phe Cys Ile Trp
    210                 215                 220

Lys Gly Val Lys Ser Thr Gly Lys Val Val Tyr Phe Thr Ala Thr Phe
225                 230                 235                 240

Pro Tyr Leu Met Leu Val Val Leu Leu Ile Arg Gly Val Thr Leu Pro
                245                 250                 255

Gly Ala Ala Gln Gly Ile Gln Phe Tyr Leu Tyr Pro Asn Ile Thr Arg
            260                 265                 270

Leu Trp Asp Pro Gln Val Trp Met Asp Ala Gly Thr Gln Ile Phe Phe
        275                 280                 285

Ser Phe Ala Ile Cys Leu Gly Cys Leu Thr Ala Leu Gly Ser Tyr Asn
    290                 295                 300

Lys Tyr His Asn Asn Cys Tyr Arg Asp Cys Val Ala Leu Cys Ile Leu
305                 310                 315                 320

Asn Ser Ser Thr Ser Phe Val Ala Gly Phe Ala Ile Phe Ser Ile Leu
                325                 330                 335
```

Gly Phe Met Ser Gln Glu Gln Gly Val Pro Ile Ser Glu Val Ala Glu
340 345 350

Ser Gly Pro Gly Leu Ala Phe Ile Ala Tyr Pro Arg Ala Val Val Met
355 360 365

Leu Pro Phe Ser Pro Leu Trp Ala Cys Cys Phe Phe Phe Met Val Val
370 375 380

Leu Leu Gly Leu Asp Ser Gln Phe Val Cys Val Glu Ser Leu Val Thr
385 390 395 400

Ala Leu Val Asp Met Tyr Pro Arg Val Phe Arg Lys Lys Asn Arg Arg
405 410 415

Glu Ile Leu Ile Leu Ile Val Ser Val Val Ser Phe Phe Ile Gly Leu
420 425 430

Ile Met Leu Thr Glu Gly Gly Met Tyr Val Phe Gln Leu Phe Asp Tyr
435 440 445

Tyr Ala Ala Ser Gly Met Cys Leu Leu Phe Val Ala Ile Phe Glu Ser
450 455 460

Leu Cys Val Ala Trp Val Tyr Gly Ala Ser Arg Phe Tyr Asp Asn Ile
465 470 475 480

Glu Asp Met Ile Gly Tyr Lys Pro Trp Pro Leu Ile Lys Tyr Cys Trp
485 490 495

Leu Phe Phe Thr Pro Ala Val Cys Leu Ala Thr Phe Leu Phe Ser Leu
500 505 510

Ile Lys Tyr Thr Pro Leu Thr Tyr Asn Lys Lys Tyr Thr Tyr Pro Trp
515 520 525

Trp Gly Asp Ala Leu Gly Trp Leu Leu Ala Leu Ser Ser Met Val Cys
530 535 540

Ile Pro Ala Trp Ser Ile Tyr Lys Leu Arg Thr Leu Lys Gly Pro Leu
545 550 555 560

Arg Glu Arg Leu Arg Gln Leu Val Cys Pro Ala Glu Asp Leu Pro Gln
565 570 575

Lys Ser Gln Pro Glu Leu Thr Ser Pro Ala Thr Pro Met Thr Ser Leu
580 585 590

Leu Arg Leu Thr Glu Leu Glu Ser Asn Cys
595 600

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1938 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(v) FRAGMENT TYPE: N-terminal

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: rat brain
(B) CLONE: rB8b

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 16..1897
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GGCGGCAGGG CGGCC ATG ACT GCG GAG CAA GCG CTG CCC CTG GGC AAC GGG    51
                 Met Thr Ala Glu Gln Ala Leu Pro Leu Gly Asn Gly
                  1               5                  10

AAG GCG GCC GAG GAG GCG CGA GGG TCC GAG GCG CTG GGC GGC GGC GGC     99
Lys Ala Ala Glu Glu Ala Arg Gly Ser Glu Ala Leu Gly Gly Gly Gly
         15                  20                  25

GGG GGC GCG GCG GGG ACG CGC GAG GCG CGC GAC AAG GCG GTC CAC GAG    147
Gly Gly Ala Ala Gly Thr Arg Glu Ala Arg Asp Lys Ala Val His Glu
     30                  35                  40

CGC GGT CAC TGG AAC AAC AAG GTG GAG TTC GTG TTG AGC GTA GCG GGA    195
Arg Gly His Trp Asn Asn Lys Val Glu Phe Val Leu Ser Val Ala Gly
 45                  50                  55                  60

GAG ATC ATC GGT CTG GGC AAC GTG TGG CGC TTC CCC TAC CTG TGC TAC    243
Glu Ile Ile Gly Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr
                 65                  70                  75

AAG AAC GGC GGA GGG GCA TTC CTG ATT CCT TAC GTG GTG TTT TTC ATC    291
Lys Asn Gly Gly Gly Ala Phe Leu Ile Pro Tyr Val Val Phe Phe Ile
             80                  85                  90

TGC TGT GGA ATC CCC GTC TTC TTC CTG GAA ACG GCT CTG GGG CAG TTC    339
Cys Cys Gly Ile Pro Val Phe Phe Leu Glu Thr Ala Leu Gly Gln Phe
         95                 100                 105

ACG AGC GAG GGC GGC ATC ACG TGC TGG AGG AGA GTC TGT CCT TTA TTT    387
Thr Ser Glu Gly Gly Ile Thr Cys Trp Arg Arg Val Cys Pro Leu Phe
    110                 115                 120

GAA GGC ATC GGC TAT GCA ACA CAG GTG ATC GAG GCG CAT CTC AAT GTC    435
Glu Gly Ile Gly Tyr Ala Thr Gln Val Ile Glu Ala His Leu Asn Val
125                 130                 135                 140

TAC TAC ATC ATC ATC CTG GCG TGG GCC ATC TTC TAC TTA AGC AAC TGC    483
Tyr Tyr Ile Ile Ile Leu Ala Trp Ala Ile Phe Tyr Leu Ser Asn Cys
                145                 150                 155

TTC ACC ACC GAG CTC CCC TGG GCC ACC TGT GGG CAT GAG TGG AAC ACA    531
Phe Thr Thr Glu Leu Pro Trp Ala Thr Cys Gly His Glu Trp Asn Thr
            160                 165                 170

GAG AAA TGT GTG GAG TTC CAG AAG CTG AAC TTC AGC AAC TAC AGT CAT    579
Glu Lys Cys Val Glu Phe Gln Lys Leu Asn Phe Ser Asn Tyr Ser His
        175                 180                 185
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GTG TCC CTG CAG AAC GCA ACC TCC CCG GTC ATG GAG TTC TGG GAA CGC 627
Val Ser Leu Gln Asn Ala Thr Ser Pro Val Met Glu Phe Trp Glu Arg
190 195 200

CGG GTC TTG GCT ATA TCT GAT GGC ATT GAA CAC ATC GGG AAC CTC CGA 675
Arg Val Leu Ala Ile Ser Asp Gly Ile Glu His Ile Gly Asn Leu Arg
205 210 215 220

TGG GAG CTG GCA CTG TGT CTC CTG GCG GCT TGG ACC ATC TGC TAC TTC 723
Trp Glu Leu Ala Leu Cys Leu Leu Ala Ala Trp Thr Ile Cys Tyr Phe
225 230 235

TGC ATC TGG AAG GGT ACG AAG TCA ACT GGA AAG GTC GTG TAT GTC ACT 771
Cys Ile Trp Lys Gly Thr Lys Ser Thr Gly Lys Val Val Tyr Val Thr
240 245 250

GCA ACC TTC CCC TAC ATC ATG CTG CTG ATC CTC CTG ATC CGA GGG GTC 819
Ala Thr Phe Pro Tyr Ile Met Leu Leu Ile Leu Leu Ile Arg Gly Val
255 260 265

ACG TTG CCG GGT GCC TCG GAA GGC ATC AAG TTC TAC CTG TAC CCT GAC 867
Thr Leu Pro Gly Ala Ser Glu Gly Ile Lys Phe Tyr Leu Tyr Pro Asp
270 275 280

CTC TCC CGG CTC TCT GAT CCA CAG GTG TGG GTG GAT GCT GGG ACG CAG 915
Leu Ser Arg Leu Ser Asp Pro Gln Val Trp Val Asp Ala Gly Thr Gln
285 290 295 300

ATC TTT TTC TCC TAT GCC ATC TGC CTG GGC TGC CTG ACC GCT CTG GGG 963
Ile Phe Phe Ser Tyr Ala Ile Cys Leu Gly Cys Leu Thr Ala Leu Gly
305 310 315

AGT TAC AAC AAC TAT AAC AAC AAC TGC TAC AGG GAC TGT ATT ATG CTC 1011
Ser Tyr Asn Asn Tyr Asn Asn Asn Cys Tyr Arg Asp Cys Ile Met Leu
320 325 330

TGC TGT CTG AAC AGT GGC ACC AGC TTC GTG GCT GGG TTT GCT ATC TTC 1059
Cys Cys Leu Asn Ser Gly Thr Ser Phe Val Ala Gly Phe Ala Ile Phe
335 340 345

TCA GTC CTG GGC TTC ATG GCG TAC GAG CAG GGC GTG CCT ATT GCT GAG 1107
Ser Val Leu Gly Phe Met Ala Tyr Glu Gln Gly Val Pro Ile Ala Glu
350 355 360

GTG GCA GAA TCA GGT CCT GGA CTG GCT TTC ATC GCC TAC CCC AAG GCT 1155
Val Ala Glu Ser Gly Pro Gly Leu Ala Phe Ile Ala Tyr Pro Lys Ala
365 370 375 380

GTC ACT ATG ATG CCC CTG TCC CCA TTG TGG GCC ACC CTG TTC TTC ATG 1203
Val Thr Met Met Pro Leu Ser Pro Leu Trp Ala Thr Leu Phe Phe Met
385 390 395

ATG CTC ATC TTC CTG GGC CTG GAC AGT CAG TTT GTG TGT GTG GAG AGC 1251
Met Leu Ile Phe Leu Gly Leu Asp Ser Gln Phe Val Cys Val Glu Ser
400 405 410

CTT GTG ACA GCC GTG GTT GAC ATG TAC CCC AAG GTC TTC CGG CGG GGC 1299
Leu Val Thr Ala Val Val Asp Met Tyr Pro Lys Val Phe Arg Arg Gly
415 420 425

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TAC CGG CGA GAA CTG CTC ATC CTG GCC CTG TCC ATT GTC TCT TAT TTC       1347
Tyr Arg Arg Glu Leu Leu Ile Leu Ala Leu Ser Ile Val Ser Tyr Phe
    430                 435                     440

CTA GGC CTG GTG ATG CTG ACA GAG GGA GGC ATG TAC ATT TTC CAG CTT       1395
Leu Gly Leu Val Met Leu Thr Glu Gly Gly Met Tyr Ile Phe Gln Leu
445                 450                     455                 460

TTT GAC TCA TAC GCC GCC AGT GGC ATG TGC TTG CTC TTC GTG GCC ATC       1443
Phe Asp Ser Tyr Ala Ala Ser Gly Met Cys Leu Leu Phe Val Ala Ile
                465                     470                 475

TTT GAG TGT GTC TGC ATC GGC TGG GTG TAT GGA AGT AAC AGG TTC TAT       1491
Phe Glu Cys Val Cys Ile Gly Trp Val Tyr Gly Ser Asn Arg Phe Tyr
            480                     485                 490

GAC AAT ATT GAG GAC ATG ATT GGA TAC CGG CCA CTG TCA CTC ATC AAG       1539
Asp Asn Ile Glu Asp Met Ile Gly Tyr Arg Pro Leu Ser Leu Ile Lys
        495                     500                 505

TGG TGC TGG AAA GTT GTG ACC CCT GGG ATC TGT GCG GGC ATC TTC ATC       1587
Trp Cys Trp Lys Val Val Thr Pro Gly Ile Cys Ala Gly Ile Phe Ile
    510                     515                 520

TTC TTT CTG GTC AAG TAC AAG CCG CTC AAG TAC AAC AAT GTG TAC ACA       1635
Phe Phe Leu Val Lys Tyr Lys Pro Leu Lys Tyr Asn Asn Val Tyr Thr
525                 530                     535                 540

TAT CCT GCT TGG GGC TAC GGC ATT GGC TGG CTC ATG GCT CTG TCC TCC       1683
Tyr Pro Ala Trp Gly Tyr Gly Ile Gly Trp Leu Met Ala Leu Ser Ser
                545                     550                 555

ATG CTG TGC ATC CCG CTC TGG ATC TTC ATC AAG CTG TGG AAG ACA GAG       1731
Met Leu Cys Ile Pro Leu Trp Ile Phe Ile Lys Leu Trp Lys Thr Glu
            560                     565                 570

GGC ACC CTG CCC GAG AAA TTA CAG AAG TTG ACA GTC CCC AGC GCT GAT       1779
Gly Thr Leu Pro Glu Lys Leu Gln Lys Leu Thr Val Pro Ser Ala Asp
        575                 580                     585

CTG AAA ATG AGG GGC AAG CTT GGG GCC AGC CCA CGG ATG GTG ACC GTT       1827
Leu Lys Met Arg Gly Lys Leu Gly Ala Ser Pro Arg Met Val Thr Val
    590                 595                     600

AAT GAC TGT GAG GCC AAG GTC AAA GGC GAC GGT ACC ATC TCT GCC ATC       1875
Asn Asp Cys Glu Ala Lys Val Lys Gly Asp Gly Thr Ile Ser Ala Ile
605                 610                     615                 620

ACA GAG AAG GAG ACG CAC TTC T GATCCCCGCC AGCCACTTGG ATGTGTCTCC        1927
Thr Glu Lys Glu Thr His Phe
                625

AGCCTTCCTT C                                                          1938
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 627 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Thr Ala Glu Gln Ala Leu Pro Leu Gly Asn Gly Lys Ala Ala Glu
 1               5                  10                  15

Glu Ala Arg Gly Ser Glu Ala Leu Gly Gly Gly Gly Gly Gly Ala Ala
            20                  25                  30

Gly Thr Arg Glu Ala Arg Asp Lys Ala Val His Glu Arg Gly His Trp
        35                  40                  45

Asn Asn Lys Val Glu Phe Val Leu Ser Val Ala Gly Glu Ile Ile Gly
    50                  55                  60

Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Lys Asn Gly Gly
65                  70                  75                  80

Gly Ala Phe Leu Ile Pro Tyr Val Val Phe Phe Ile Cys Cys Gly Ile
                85                  90                  95

Pro Val Phe Phe Leu Glu Thr Ala Leu Gly Gln Phe Thr Ser Glu Gly
            100                 105                 110

Gly Ile Thr Cys Trp Arg Arg Val Cys Pro Leu Phe Glu Gly Ile Gly
        115                 120                 125

Tyr Ala Thr Gln Val Ile Glu Ala His Leu Asn Val Tyr Tyr Ile Ile
    130                 135                 140

Ile Leu Ala Trp Ala Ile Phe Tyr Leu Ser Asn Cys Phe Thr Thr Glu
145                 150                 155                 160

Leu Pro Trp Ala Thr Cys Gly His Glu Trp Asn Thr Glu Lys Cys Val
                165                 170                 175

Glu Phe Gln Lys Leu Asn Phe Ser Asn Tyr Ser His Val Ser Leu Gln
            180                 185                 190

Asn Ala Thr Ser Pro Val Met Glu Phe Trp Glu Arg Arg Val Leu Ala
        195                 200                 205

Ile Ser Asp Gly Ile Glu His Ile Gly Asn Leu Arg Trp Glu Leu Ala
    210                 215                 220

Leu Cys Leu Leu Ala Ala Trp Thr Ile Cys Tyr Phe Cys Ile Trp Lys
225                 230                 235                 240

Gly Thr Lys Ser Thr Gly Lys Val Val Tyr Val Thr Ala Thr Phe Pro
                245                 250                 255

Tyr Ile Met Leu Leu Ile Leu Leu Ile Arg Gly Val Thr Leu Pro Gly
            260                 265                 270

Ala Ser Glu Gly Ile Lys Phe Tyr Leu Tyr Pro Asp Leu Ser Arg Leu
        275                 280                 285

Ser Asp Pro Gln Val Trp Val Asp Ala Gly Thr Gln Ile Phe Phe Ser
    290                 295                 300
```

Tyr Ala Ile Cys Leu Gly Cys Leu Thr Ala Leu Gly Ser Tyr Asn Asn
305 310 315 320

Tyr Asn Asn Asn Cys Tyr Arg Asp Cys Ile Met Leu Cys Cys Leu Asn
325 330 335

Ser Gly Thr Ser Phe Val Ala Gly Phe Ala Ile Phe Ser Val Leu Gly
340 345 350

Phe Met Ala Tyr Glu Gln Gly Val Pro Ile Ala Glu Val Ala Glu Ser
355 360 365

Gly Pro Gly Leu Ala Phe Ile Ala Tyr Pro Lys Ala Val Thr Met Met
370 375 380

Pro Leu Ser Pro Leu Trp Ala Thr Leu Phe Phe Met Met Leu Ile Phe
385 390 395 400

Leu Gly Leu Asp Ser Gln Phe Val Cys Val Glu Ser Leu Val Thr Ala
405 410 415

Val Val Asp Met Tyr Pro Lys Val Phe Arg Arg Gly Tyr Arg Arg Glu
420 425 430

Leu Leu Ile Leu Ala Leu Ser Ile Val Ser Tyr Phe Leu Gly Leu Val
435 440 445

Met Leu Thr Glu Gly Gly Met Tyr Ile Phe Gln Leu Phe Asp Ser Tyr
450 455 460

Ala Ala Ser Gly Met Cys Leu Leu Phe Val Ala Ile Phe Glu Cys Val
465 470 475 480

Cys Ile Gly Trp Val Tyr Gly Ser Asn Arg Phe Tyr Asp Asn Ile Glu
485 490 495

Asp Met Ile Gly Tyr Arg Pro Leu Ser Leu Ile Lys Trp Cys Trp Lys
500 505 510

Val Val Thr Pro Gly Ile Cys Ala Gly Ile Phe Ile Phe Phe Leu Val
515 520 525

Lys Tyr Lys Pro Leu Lys Tyr Asn Asn Val Tyr Thr Tyr Pro Ala Trp
530 535 540

Gly Tyr Gly Ile Gly Trp Leu Met Ala Leu Ser Ser Met Leu Cys Ile
545 550 555 560

Pro Leu Trp Ile Phe Ile Lys Leu Trp Lys Thr Glu Gly Thr Leu Pro
565 570 575

Glu Lys Leu Gln Lys Leu Thr Val Pro Ser Ala Asp Leu Lys Met Arg
580 585 590

Gly Lys Leu Gly Ala Ser Pro Arg Met Val Thr Val Asn Asp Cys Glu
595 600 605

Ala Lys Val Lys Gly Asp Gly Thr Ile Ser Ala Ile Thr Glu Lys Glu
610 615 620

Thr His Phe
625

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2093 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Taurine

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: rat brain
(B) CLONE: rB16a

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 127..1989
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCCAACGCCG CGATCGCCGC CAATCCCGCC AGCCTCGGGC CGGGCCATCC GCTGTGGGCT 60

TAGCCACCCA GATGCAGAGC CAGTGCCACA GCCTCTTCAG AGGAGCCTCT CAAGCAAAAC 120

GAGGAG ATG GCC ACC AAG GAG AAG CTT CAA TGT CTG AAA GAC TTC CAC 168
Met Ala Thr Lys Glu Lys Leu Gln Cys Leu Lys Asp Phe His
1 5 10

AAA GAC ATC CTG AAG CCT TCT CCA GGG AAG AGC CCA GGC ACG CGG CCT 216
Lys Asp Ile Leu Lys Pro Ser Pro Gly Lys Ser Pro Gly Thr Arg Pro
15 20 25 30

GAG GAT GAG GCT GAT GGG AAG CCC CCT CAG AGG GAG AAG TGG TCC AGC 264
Glu Asp Glu Ala Asp Gly Lys Pro Pro Gln Arg Glu Lys Trp Ser Ser
35 40 45

AAG ATC GAC TTT GTG CTG TCT GTG GCC GGA GGC TTC GTG GGT TTG GGC 312
Lys Ile Asp Phe Val Leu Ser Val Ala Gly Gly Phe Val Gly Leu Gly
50 55 60

AAT GTC TGG CGT TTC CCG TAC CTC TGC TAC AAA AAT GGT GGA GGT GCA 360
Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Lys Asn Gly Gly Gly Ala
65 70 75

TTC CTC ATA CCG TAT TTT ATT TTC CTG TTT GGG AGC GGC CTG CCT GTG 408
Phe Leu Ile Pro Tyr Phe Ile Phe Leu Phe Gly Ser Gly Leu Pro Val
80 85 90

TTT TTC CTG GAG GTC ATC ATA GGC CAG TAC ACC TCA GAA GGG GGC ATC 456
Phe Phe Leu Glu Val Ile Ile Gly Gln Tyr Thr Ser Glu Gly Gly Ile
95 100 105 110

```
ACC TGC TGG GAG AAG ATC TGC CCC TTG TTC TCT GGC ATT GGC TAC GCG      504
Thr Cys Trp Glu Lys Ile Cys Pro Leu Phe Ser Gly Ile Gly Tyr Ala
                115                 120                 125

TCC ATC GTC ATC GTG TCC CTC CTG AAT GTG TAC TAC ATC GTC ATC CTG      552
Ser Ile Val Ile Val Ser Leu Leu Asn Val Tyr Tyr Ile Val Ile Leu
            130                 135                 140

GCC TGG GCC ACA TAC TAC CTA TTC CAG TCT TTC CAG AAG GAT CTT CCC      600
Ala Trp Ala Thr Tyr Tyr Leu Phe Gln Ser Phe Gln Lys Asp Leu Pro
        145                 150                 155

TGG GCC CAC TGC AAC CAT AGC TGG AAC ACG CCA CAG TGC ATG GAG GAC      648
Trp Ala His Cys Asn His Ser Trp Asn Thr Pro Gln Cys Met Glu Asp
    160                 165                 170

ACC CTG CGT AGG AAC GAG AGT CAC TGG GTC TCC CTT AGC GCC GCC AAC      696
Thr Leu Arg Arg Asn Glu Ser His Trp Val Ser Leu Ser Ala Ala Asn
175                 180                 185                 190

TTC ACT TCG CCT GTG ATC GAG TTC TGG GAG CGC AAC GTG CTC AGC CTG      744
Phe Thr Ser Pro Val Ile Glu Phe Trp Glu Arg Asn Val Leu Ser Leu
                195                 200                 205

TCC TCC GGA ATC GAC CAC CCA GGC AGT CTG AAA TGG GAC CTC GCG CTC      792
Ser Ser Gly Ile Asp His Pro Gly Ser Leu Lys Trp Asp Leu Ala Leu
            210                 215                 220

TGC CTC CTC TTA GTC TGG CTC GTC TGT TTT TTC TGC ATC TGG AAG GGT      840
Cys Leu Leu Leu Val Trp Leu Val Cys Phe Phe Cys Ile Trp Lys Gly
        225                 230                 235

GTT CGG TCC ACA GGC AAG GTT GTC TAC TTC ACT GCT ACT TTC CCG TTT      888
Val Arg Ser Thr Gly Lys Val Val Tyr Phe Thr Ala Thr Phe Pro Phe
    240                 245                 250

GCC ATG CTT CTG GTG CTG CTG GTC CGT GGA CTG ACC CTG CCA GGT GCT      936
Ala Met Leu Leu Val Leu Leu Val Arg Gly Leu Thr Leu Pro Gly Ala
255                 260                 265                 270

GGT GAA GGC ATC AAA TTC TAC CTG TAC CCT AAC ATC AGC CGC CTT GAG      984
Gly Glu Gly Ile Lys Phe Tyr Leu Tyr Pro Asn Ile Ser Arg Leu Glu
                275                 280                 285

GAC CCA CAG GTG TGG ATC GAC GCT GGA ACT CAG ATA TTC TTT TCC TAC     1032
Asp Pro Gln Val Trp Ile Asp Ala Gly Thr Gln Ile Phe Phe Ser Tyr
            290                 295                 300

GCT ATC TGC CTG GGG GCC ATG ACC TCA CTG GGA AGC TAT AAC AAG TAC     1080
Ala Ile Cys Leu Gly Ala Met Thr Ser Leu Gly Ser Tyr Asn Lys Tyr
        305                 310                 315

AAG TAT AAC TCG TAC AGG GAC TGT ATG CTG CTG GGA TGC CTG AAC AGT     1128
Lys Tyr Asn Ser Tyr Arg Asp Cys Met Leu Leu Gly Cys Leu Asn Ser
    320                 325                 330

GGT ACC AGT TTT GTG TCT GGC TTC GCA ATT TTT TCC ATC CTG GGC TTC     1176
Gly Thr Ser Phe Val Ser Gly Phe Ala Ile Phe Ser Ile Leu Gly Phe
335                 340                 345                 350
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ATG GCA CAA GAG CAA GGG GTG GAC ATT GCT GAT GTG GCT GAG TCA GGT     1224
Met Ala Gln Glu Gln Gly Val Asp Ile Ala Asp Val Ala Glu Ser Gly
                355                 360                 365

CCT GGC TTG GCC TTC ATT GCC TAC CCA AAA GCT GTG ACC ATG ATG CCG     1272
Pro Gly Leu Ala Phe Ile Ala Tyr Pro Lys Ala Val Thr Met Met Pro
            370                 375                 380

CTG CCC ACC TTT TGG TCC ATT CTG TTT TTT ATT ATG CTC CTC TTG CTT     1320
Leu Pro Thr Phe Trp Ser Ile Leu Phe Phe Ile Met Leu Leu Leu Leu
        385                 390                 395

GGA CTG GAC AGC CAG TTT GTT GAA GTC GAA GGA CAG ATC ACA TCC TTG     1368
Gly Leu Asp Ser Gln Phe Val Glu Val Glu Gly Gln Ile Thr Ser Leu
    400                 405                 410

GTT GAT CTT TAC CCG TCC TTC CTA AGG AAG GGT TAT CGT CGG GAA ATC     1416
Val Asp Leu Tyr Pro Ser Phe Leu Arg Lys Gly Tyr Arg Arg Glu Ile
415                 420                 425                 430

TTC ATT GCC ATC GTG TGC AGC ATC AGC TAC CTG CTG GGG CTG ACG ATG     1464
Phe Ile Ala Ile Val Cys Ser Ile Ser Tyr Leu Leu Gly Leu Thr Met
                435                 440                 445

GTG ACG GAG GGT GGC ATG TAT GTG TTT CAA CTC TTT GAC TAC TAT GCA     1512
Val Thr Glu Gly Gly Met Tyr Val Phe Gln Leu Phe Asp Tyr Tyr Ala
            450                 455                 460

GCT AGT GGT GTA TGC CTT TTG TGG GTC GCA TTC TTT GAA TGT TTT GTT     1560
Ala Ser Gly Val Cys Leu Leu Trp Val Ala Phe Phe Glu Cys Phe Val
        465                 470                 475

ATT GCC TGG ATA TAT GGC GGT GAT AAC TTA TAT GAC GGT ATT GAG GAC     1608
Ile Ala Trp Ile Tyr Gly Gly Asp Asn Leu Tyr Asp Gly Ile Glu Asp
    480                 485                 490

ATG ATC GGC TAT CGG CCT GGA CCC TGG ATG AAG TAC AGC TGG GCT GTC     1656
Met Ile Gly Tyr Arg Pro Gly Pro Trp Met Lys Tyr Ser Trp Ala Val
495                 500                 505                 510

ATC ACT CCA GCT CTC TGT GTT GGA TGT TTC ATC TTC TCT CTC GTC AAG     1704
Ile Thr Pro Ala Leu Cys Val Gly Cys Phe Ile Phe Ser Leu Val Lys
                515                 520                 525

TAT GTA CCC CTG ACC TAC AAC AAA GTC TAC CGG TAC CCT GAT TGG GCA     1752
Tyr Val Pro Leu Thr Tyr Asn Lys Val Tyr Arg Tyr Pro Asp Trp Ala
            530                 535                 540

ATC GGG CTG GGC TGG GGC CTG GCC CTT TCC TCC ATG GTG TGT ATC CCC     1800
Ile Gly Leu Gly Trp Gly Leu Ala Leu Ser Ser Met Val Cys Ile Pro
        545                 550                 555

TTG GTC ATT GTC ATC CTC CTC TGC CGG ACG GAG GGA CCG CTC CGC GTG     1848
Leu Val Ile Val Ile Leu Leu Cys Arg Thr Glu Gly Pro Leu Arg Val
    560                 565                 570

AGA ATC AAA TAC CTG ATA ACC CCC AGG GAG CCC AAC CGC TGG GCT GTG     1896
Arg Ile Lys Tyr Leu Ile Thr Pro Arg Glu Pro Asn Arg Trp Ala Val
575                 580                 585                 590
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GAG CGT GAA GGG GCT ACG CCC TTT CAC TCC AGA GCA ACC CTC ATG AAC     1944
Glu Arg Glu Gly Ala Thr Pro Phe His Ser Arg Ala Thr Leu Met Asn
                595                 600                 605

GGT GCA CTC ATG AAA CCC AGT CAC GTC ATT GTG GAG ACC ATG ATG         1989
Gly Ala Leu Met Lys Pro Ser His Val Ile Val Glu Thr Met Met
            610                 615                 620

TGAGGTCCGG GCTGTGTGAC CGGCGCCGCT TTCCTGCCGT TTACTAACCT TAGATTCTCC   2049

TAGGACCAGG TTTACAGAGC TTTATATTTG TACTAGGATT TTTT                    2093
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 621 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Thr Lys Glu Lys Leu Gln Cys Leu Lys Asp Phe His Lys Asp
 1               5                  10                  15

Ile Leu Lys Pro Ser Pro Gly Lys Ser Pro Gly Thr Arg Pro Glu Asp
            20                  25                  30

Glu Ala Asp Gly Lys Pro Pro Gln Arg Glu Lys Trp Ser Ser Lys Ile
        35                  40                  45

Asp Phe Val Leu Ser Val Ala Gly Gly Phe Val Gly Leu Gly Asn Val
    50                  55                  60

Trp Arg Phe Pro Tyr Leu Cys Tyr Lys Asn Gly Gly Gly Ala Phe Leu
65                  70                  75                  80

Ile Pro Tyr Phe Ile Phe Leu Phe Gly Ser Gly Leu Pro Val Phe Phe
                85                  90                  95

Leu Glu Val Ile Ile Gly Gln Tyr Thr Ser Glu Gly Gly Ile Thr Cys
            100                 105                 110

Trp Glu Lys Ile Cys Pro Leu Phe Ser Gly Ile Gly Tyr Ala Ser Ile
        115                 120                 125

Val Ile Val Ser Leu Leu Asn Val Tyr Tyr Ile Val Ile Leu Ala Trp
    130                 135                 140

Ala Thr Tyr Tyr Leu Phe Gln Ser Phe Gln Lys Asp Leu Pro Trp Ala
145                 150                 155                 160

His Cys Asn His Ser Trp Asn Thr Pro Gln Cys Met Glu Asp Thr Leu
                165                 170                 175

Arg Arg Asn Glu Ser His Trp Val Ser Leu Ser Ala Ala Asn Phe Thr
            180                 185                 190

Ser Pro Val Ile Glu Phe Trp Glu Arg Asn Val Leu Ser Leu Ser Ser
        195                 200                 205
```

Gly Ile Asp His Pro Gly Ser Leu Lys Trp Asp Leu Ala Leu Cys Leu
210 215 220

Leu Leu Val Trp Leu Val Cys Phe Phe Cys Ile Trp Lys Gly Val Arg
225 230 235 240

Ser Thr Gly Lys Val Val Tyr Phe Thr Ala Thr Phe Pro Phe Ala Met
245 250 255

Leu Leu Val Leu Leu Val Arg Gly Leu Thr Leu Pro Gly Ala Gly Glu
260 265 270

Gly Ile Lys Phe Tyr Leu Tyr Pro Asn Ile Ser Arg Leu Glu Asp Pro
275 280 285

Gln Val Trp Ile Asp Ala Gly Thr Gln Ile Phe Phe Ser Tyr Ala Ile
290 295 300

Cys Leu Gly Ala Met Thr Ser Leu Gly Ser Tyr Asn Lys Tyr Lys Tyr
305 310 315 320

Asn Ser Tyr Arg Asp Cys Met Leu Leu Gly Cys Leu Asn Ser Gly Thr
325 330 335

Ser Phe Val Ser Gly Phe Ala Ile Phe Ser Ile Leu Gly Phe Met Ala
340 345 350

Gln Glu Gln Gly Val Asp Ile Ala Asp Val Ala Glu Ser Gly Pro Gly
355 360 365

Leu Ala Phe Ile Ala Tyr Pro Lys Ala Val Thr Met Met Pro Leu Pro
370 375 380

Thr Phe Trp Ser Ile Leu Phe Phe Ile Met Leu Leu Leu Leu Gly Leu
385 390 395 400

Asp Ser Gln Phe Val Glu Val Glu Gly Gln Ile Thr Ser Leu Val Asp
405 410 415

Leu Tyr Pro Ser Phe Leu Arg Lys Gly Tyr Arg Arg Glu Ile Phe Ile
420 425 430

Ala Ile Val Cys Ser Ile Ser Tyr Leu Leu Gly Leu Thr Met Val Thr
435 440 445

Glu Gly Gly Met Tyr Val Phe Gln Leu Phe Asp Tyr Tyr Ala Ala Ser
450 455 460

Gly Val Cys Leu Leu Trp Val Ala Phe Phe Glu Cys Phe Val Ile Ala
465 470 475 480

Trp Ile Tyr Gly Gly Asp Asn Leu Tyr Asp Gly Ile Glu Asp Met Ile
485 490 495

Gly Tyr Arg Pro Gly Pro Trp Met Lys Tyr Ser Trp Ala Val Ile Thr
500 505 510

Pro Ala Leu Cys Val Gly Cys Phe Ile Phe Ser Leu Val Lys Tyr Val
515 520 525

Pro Leu Thr Tyr Asn Lys Val Tyr Arg Tyr Pro Asp Trp Ala Ile Gly
530 535 540

```
Leu Gly Trp Gly Leu Ala Leu Ser Ser Met Val Cys Ile Pro Leu Val
545                 550                 555                 560

Ile Val Ile Leu Leu Cys Arg Thr Glu Gly Pro Leu Arg Val Arg Ile
                565                 570                 575

Lys Tyr Leu Ile Thr Pro Arg Glu Pro Asn Arg Trp Ala Val Glu Arg
            580                 585                 590

Glu Gly Ala Thr Pro Phe His Ser Arg Ala Thr Leu Met Asn Gly Ala
        595                 600                 605

Leu Met Lys Pro Ser His Val Ile Val Glu Thr Met Met
    610                 615                 620
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1051 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: human heart, human brain
(B) CLONE: hHE7a,hS3a

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..739
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CTG GCT TTC ATC GCT TAC CCG CGG GCT GTG GTG ATG CTG CCC TTC TCT       48
Leu Ala Phe Ile Ala Tyr Pro Arg Ala Val Val Met Leu Pro Phe Ser
  1               5                  10                  15

CCT CTC TGG GCC TGC TGT TTC TTC TTC ATG GTC GTT CTC CTG GGA CTG       96
Pro Leu Trp Ala Cys Cys Phe Phe Phe Met Val Val Leu Leu Gly Leu
            20                  25                  30

GAT AGC CAG TTT GTG TGT GTA GAA AGC CTG GTG ACA GCG CTG GTG GAC      144
Asp Ser Gln Phe Val Cys Val Glu Ser Leu Val Thr Ala Leu Val Asp
        35                  40                  45

ATG TAC CCT CAC GTG TTC CGC AAG AAG AAC CGG AGG GAA GTC CTC ATC      192
Met Tyr Pro His Val Phe Arg Lys Lys Asn Arg Arg Glu Val Leu Ile
    50                  55                  60

CTT GGA GTA TCT GTC GTC TCC TTC CTT GTG GGG CTG ATC ATG CTC ACA      240
Leu Gly Val Ser Val Val Ser Phe Leu Val Gly Leu Ile Met Leu Thr
 65                  70                  75                  80
```

GAG GGC GGA ATG TAC GTG TTC CAG CTC TTT GAC TAC TAT GCG GCC AGT 288
Glu Gly Gly Met Tyr Val Phe Gln Leu Phe Asp Tyr Tyr Ala Ala Ser
85 90 95

GGC ATG TGC CTC CTG TTC GTG GCC ATC TTC GAG TCC CTC TGT GTG GCT 336
Gly Met Cys Leu Leu Phe Val Ala Ile Phe Glu Ser Leu Cys Val Ala
100 105 110

TGG GTT TAC GGA GCC AAG CGC TTC TAC GAC AAC ATC GAA GAC ATG ATT 384
Trp Val Tyr Gly Ala Lys Arg Phe Tyr Asp Asn Ile Glu Asp Met Ile
115 120 125

GGG TAC AGG CCA TGG CCT CTT ATC AAA TAC TGT TGG CTC TTC CTC ACA 432
Gly Tyr Arg Pro Trp Pro Leu Ile Lys Tyr Cys Trp Leu Phe Leu Thr
130 135 140

CCA GCT GTG TGC ACA GCC ACC TTT CTC TTC TCC CTG ATA AAG TAC ACT 480
Pro Ala Val Cys Thr Ala Thr Phe Leu Phe Ser Leu Ile Lys Tyr Thr
145 150 155 160

CCG CTG ACC TAC AAC AAG AAG TAC ACG TAC CCG TGG TGG GGC GAT GCC 528
Pro Leu Thr Tyr Asn Lys Lys Tyr Thr Tyr Pro Trp Trp Gly Asp Ala
165 170 175

CTG GGC TGG CTC CTG GCT CTG TCC TCC ATG GTC TGC ATT CCT GCC TGG 576
Leu Gly Trp Leu Leu Ala Leu Ser Ser Met Val Cys Ile Pro Ala Trp
180 185 190

AGC CTC TAC AGA CTC GGA ACC CTC AAG GGC CCC TTC AGA GAG AGA ATC 624
Ser Leu Tyr Arg Leu Gly Thr Leu Lys Gly Pro Phe Arg Glu Arg Ile
195 200 205

CGT CAG CTC ATG TGC CCA GCC GAG GAC CTG CCC CAG CGG AAC CCA GCA 672
Arg Gln Leu Met Cys Pro Ala Glu Asp Leu Pro Gln Arg Asn Pro Ala
210 215 220

GGA CCC TCG GCT CCC GCC ACC CCC AGG ACC TCA CTG CTC AGA CTC ACA 720
Gly Pro Ser Ala Pro Ala Thr Pro Arg Thr Ser Leu Leu Arg Leu Thr
225 230 235 240

GAG CTA GAG TCT CAC TGC T AGGGGGCAGG CCCTTGGATG GTGCCTGTGT 769
Glu Leu Glu Ser His Cys
245

GCCTGGCCTT GGGGATGGCT GTGGAGGGAA CGTGGCAGAA GCAGCCCCAT GTGCTTCCCT 829

GCCCCCGACC TGGAGTGGAT AAGACAAGAG GGGTATTTTG GAGTCCACCT GCTGAGCTGG 889

AGGCCTCCCA CTGCAACTTT TCAGCTCAGG GGTTGTTGAA CAGATGTGAA AGGCCAGTGC 949

CAAGAGTGTC CCTCTGAGAC CCTTGGGAAG CTGGGTGGGG GCTGGTAGGT GGGGCGAGAC 1009

TTGCTGGCTT CGGGCCCTCT CATCCTTCAT TCCATTAAAT CC 1051

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 246 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Leu Ala Phe Ile Ala Tyr Pro Arg Ala Val Val Met Leu Pro Phe Ser
 1               5                  10                  15

Pro Leu Trp Ala Cys Cys Phe Phe Phe Met Val Val Leu Leu Gly Leu
            20                  25                  30

Asp Ser Gln Phe Val Cys Val Glu Ser Leu Val Thr Ala Leu Val Asp
        35                  40                  45

Met Tyr Pro His Val Phe Arg Lys Lys Asn Arg Arg Glu Val Leu Ile
    50                  55                  60

Leu Gly Val Ser Val Val Ser Phe Leu Val Gly Leu Ile Met Leu Thr
65                  70                  75                  80

Glu Gly Gly Met Tyr Val Phe Gln Leu Phe Asp Tyr Tyr Ala Ala Ser
                85                  90                  95

Gly Met Cys Leu Leu Phe Val Ala Ile Phe Glu Ser Leu Cys Val Ala
            100                 105                 110

Trp Val Tyr Gly Ala Lys Arg Phe Tyr Asp Asn Ile Glu Asp Met Ile
        115                 120                 125

Gly Tyr Arg Pro Trp Pro Leu Ile Lys Tyr Cys Trp Leu Phe Leu Thr
    130                 135                 140

Pro Ala Val Cys Thr Ala Thr Phe Leu Phe Ser Leu Ile Lys Tyr Thr
145                 150                 155                 160

Pro Leu Thr Tyr Asn Lys Lys Tyr Thr Tyr Pro Trp Trp Gly Asp Ala
                165                 170                 175

Leu Gly Trp Leu Leu Ala Leu Ser Ser Met Val Cys Ile Pro Ala Trp
            180                 185                 190

Ser Leu Tyr Arg Leu Gly Thr Leu Lys Gly Pro Phe Arg Glu Arg Ile
        195                 200                 205

Arg Gln Leu Met Cys Pro Ala Glu Asp Leu Pro Gln Arg Asn Pro Ala
    210                 215                 220

Gly Pro Ser Ala Pro Ala Thr Pro Arg Thr Ser Leu Leu Arg Leu Thr
225                 230                 235                 240

Glu Leu Glu Ser His Cys
                245
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1991 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: human brain
(B) CLONE: hGAT-3

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 35..1930
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
AGCCGGGCCG GCGCACGAGG CAGCCAGCGC GGCC ATG ACG GCG GAG AAG GCG      52
                                     Met Thr Ala Glu Lys Ala
                                       1               5

CTG CCC CTG GGC AAT GGG AAG GCT GCT GAG GAG GCG CGG GAG TCC GAG   100
Leu Pro Leu Gly Asn Gly Lys Ala Ala Glu Glu Ala Arg Glu Ser Glu
            10                  15                  20

GCG CCG GGT GGC GGC TGC AGC AGC GGG GGC GCG GCG CCC GCG CGC CAC   148
Ala Pro Gly Gly Gly Cys Ser Ser Gly Gly Ala Ala Pro Ala Arg His
        25                  30                  35

CCG CGC GTC AAG CGC GAC AAG GCG GTC CAC GAG CGC GGC CAC TGG AAC   196
Pro Arg Val Lys Arg Asp Lys Ala Val His Glu Arg Gly His Trp Asn
    40                  45                  50

AAC AAG GTG GAG TTC GTG CTG AGC GTG GCC GGG GAG ATC ATT GGG CTG   244
Asn Lys Val Glu Phe Val Leu Ser Val Ala Gly Glu Ile Ile Gly Leu
55                  60                  65                  70

GGC AAC GTG TGG CGC TTC CCC TAC CTG TGC TAC AAG AAC GGA GGA GGG   292
Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Lys Asn Gly Gly Gly
                75                  80                  85

GCA TTC CTG ATT CCC TAC GTG GTG TTT TTT ATT TGC TGT GGA ATT CCT   340
Ala Phe Leu Ile Pro Tyr Val Val Phe Phe Ile Cys Cys Gly Ile Pro
            90                  95                  100

GTT TTT TTC CTG GAG ACA GCT CTG GGG CAG TTC ACA AGT GAA GGT GGC   388
Val Phe Phe Leu Glu Thr Ala Leu Gly Gln Phe Thr Ser Glu Gly Gly
        105                 110                 115

ATT ACG TGT TGG AGG AAA GTT TGC CCT TTA TTT GAA GGC ATT GGC TAT   436
Ile Thr Cys Trp Arg Lys Val Cys Pro Leu Phe Glu Gly Ile Gly Tyr
    120                 125                 130

GCA ACA CAG GTG ATT GAG GCC CAT CTG AAT GTG TAC TAC ATC ATC ATC   484
Ala Thr Gln Val Ile Glu Ala His Leu Asn Val Tyr Tyr Ile Ile Ile
135                 140                 145                 150

CTG GCA TGG GCC ATT TTT TAC CTG AGC AAC TGC TTC ACT ACT GAG CTA   532
Leu Ala Trp Ala Ile Phe Tyr Leu Ser Asn Cys Phe Thr Thr Glu Leu
                155                 160                 165
```

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CCC TGG GCT ACC TGT GGG CAT GAG TGG AAC ACA GAG AAT TGT GTG GAG          580
Pro Trp Ala Thr Cys Gly His Glu Trp Asn Thr Glu Asn Cys Val Glu
            170                 175                 180

TTC CAG AAA CTG AAT GTG AGC AAC TAC AGC CAT GTG TCT CTG CAG AAT          628
Phe Gln Lys Leu Asn Val Ser Asn Tyr Ser His Val Ser Leu Gln Asn
        185                 190                 195

GCC ACC TCC CCT GTC ATG GAG TTT TGG GAG CAC CGG GTC CTG GCC ATC          676
Ala Thr Ser Pro Val Met Glu Phe Trp Glu His Arg Val Leu Ala Ile
    200                 205                 210

TCT GAC GGG ATC GAG CAC ATC GGG AAC CTT CGC TGG GAG CTG GCC TTG          724
Ser Asp Gly Ile Glu His Ile Gly Asn Leu Arg Trp Glu Leu Ala Leu
215                 220                 225                 230

TGT CTC TTG GCA GCC TGG ACC ATC TGT TAC TTC TGT ATC TGG AAG GGG          772
Cys Leu Leu Ala Ala Trp Thr Ile Cys Tyr Phe Cys Ile Trp Lys Gly
                235                 240                 245

ACC AAG TCT ACA GGA AAG GTT GTA TAC GTG ACT GCG ACA TTC CCC TAC          820
Thr Lys Ser Thr Gly Lys Val Val Tyr Val Thr Ala Thr Phe Pro Tyr
            250                 255                 260

ATC ATG CTG CTG ATC CTC CTG ATA CGA GGG GTC ACG TTG CCC GGG GCC          868
Ile Met Leu Leu Ile Leu Leu Ile Arg Gly Val Thr Leu Pro Gly Ala
        265                 270                 275

TCA GAG GGC ATC AAG TTC TAC TTG TAC CCT GAC CTC TCC CGG CTC TCC          916
Ser Glu Gly Ile Lys Phe Tyr Leu Tyr Pro Asp Leu Ser Arg Leu Ser
    280                 285                 290

GAC CCC CAG GTC TGG GTA GAT GCT GGA ACG CAG ATC TTT TTC TCC TAT          964
Asp Pro Gln Val Trp Val Asp Ala Gly Thr Gln Ile Phe Phe Ser Tyr
295                 300                 305                 310

GCC ATT TGC CTG GGC TGT CTG ACC GCT CTG GGA AGT TAT AAC AAT TAT         1012
Ala Ile Cys Leu Gly Cys Leu Thr Ala Leu Gly Ser Tyr Asn Asn Tyr
                315                 320                 325

AAC AAC AAC TGC TAC AGG GAC TGC ATC ATG CTC TGT TGC CTG AAC AGC         1060
Asn Asn Asn Cys Tyr Arg Asp Cys Ile Met Leu Cys Cys Leu Asn Ser
            330                 335                 340

GGC ACC AGC TTC GTG GCT GGG TTT GCC ATC TTC TCA GTC CTG GGT TTT         1108
Gly Thr Ser Phe Val Ala Gly Phe Ala Ile Phe Ser Val Leu Gly Phe
        345                 350                 355

ATG GCG TAC GAG CAG GGG GTA CCC ATT GCT GAG GTG GCA GAG TCA GGC         1156
Met Ala Tyr Glu Gln Gly Val Pro Ile Ala Glu Val Ala Glu Ser Gly
    360                 365                 370

CCC GGC CTG GCC TTT ATT GCG TAC CCC AAG GCG GTC ACC ATG ATG CCT         1204
Pro Gly Leu Ala Phe Ile Ala Tyr Pro Lys Ala Val Thr Met Met Pro
375                 380                 385                 390

CTC TCC CCG CTG TGG GCC ACC TTG TTC TTC ATG ATG CTC ATC TTC CTG         1252
Leu Ser Pro Leu Trp Ala Thr Leu Phe Phe Met Met Leu Ile Phe Leu
                395                 400                 405
```